Investigation into Infection Sources and Infection Routes of Bovine Spongiform Encephalopathy (BSE)

– Report on Results of Epidemiological Analysis by the BSE Epidemiological Study Group–

September 2003
BSE Technical Committee
BSE Epidemiological Study Group

September 30, 2003

Summary of the Report by the BSE Epidemiological Study Group

BSE Technical Committee
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Introduction

Japan's first case of BSE-infected cattle was discovered in September 2001, causing considerable social anxiety. In little over a month, however, a series of safety measures (including the removal of specified risk material (SRM), testing of all slaughtered cattle, and a total ban on the distribution of meat-and-bone meal) had been implemented, and measures for food safety and prevention of infection among cattle had been established.

Nevertheless, two questions remained unanswered. Namely, what was the source of infection of Japanese cases of BSE? And what route did the infection take? The Ministry of Agriculture, Forestry and Fisheries (MAFF) has conducted investigations starting from farms in which the 7 cases so far have occurred, and another survey that traced meat-and-bone meal (MBM) and other products imported from countries with known incidence of BSE. The results of these surveys have been published in the form of two reports. However, the these investigations were conducted by the administration, and did not evaluate the issue from an epidemiological aspect (an important scientific means of investigating sources of infection).

Therefore, the MAFF formed a BSE Epidemiological Study Group within the BSE Technical Committee in September 2002, and conducted epidemiological studies on the source and route of the infection. This Report collates the study results obtained through 6 meetings as well as circularized discussions.

Epidemiology is a science whose aim is to assist in establishing effective countermeasures by analyzing the patterns of outbreaks of a disease, investigating its source, and so on. It has mainly been developed in the field of infectious diseases, and uses various approaches to investigate the cause of disease outbreak. With a disease like BSE, in which the rate of incidence is low and the incubation period is long, two representative approaches are employed. One involves recording and sorting the characteristics of disease outbreaks and studying hypotheses concerning the source. The other focuses on a specific factor and traces it retrospectively.

The main work of this Study Group has been to identify, as hypotheses, all feasible sources and routes of infection for the 7 cases discovered so far, and to study the probability of each hypothesis. We quantitatively assessed risk by creating models for the infection routes considered most likely.

Unlike ordinary microbial infections, BSE has a long incubation period lasting several years. Moreover, it is technically impossible to detect very low levels of infectious agents, and the number of confirmed cases is extremely limited. Given such major constraints in conducting epidemiological analysis, the results of this epidemiological research are little more than speculation on the sources and routes of infection. Nevertheless, it is hoped that these research results will be of use in preventing future outbreaks, which is the prime objective of epidemiology.

(Kazuya Yamanouchi, Senior Researcher, Nippon Institute for Biological Science / Emeritus Professor, University of Tokyo)

1. Background

Since the first BSE-infected animal in Japan was found in September 2001, a total of seven cases have occurred so far. The MAFF has conducted investigations both from downstream, starting from the farm at which the cases occurred, and from upstream, starting from imported meat and bone meal, etc., which have failed to identify the source(s) and route(s) of infection. The ministry thus established the "BSE Epidemiological Investigation Team," consisting of experts, and performed through the team analyses and evaluations from an epidemiological point of view.

2. Epidemiological Analyses

(1) Possible hypotheses for the cause of the occurrences and their likelihood (hypothesis and testing method)

[Conducted by: Kameo Shimura, Head of the Laboratory Animal Management Section, and Toshiyuki Tsutsui, Head of the Preventive Epidemiology Research Laboratory , National Institute of Animal Health, National Agricultural Research Organization]

All possible hypotheses for the cause of the occurrence of BSE in Japan were taken up as follows and each of these hypotheses was analyzed and evaluated:

(i) Meat and bone meal contained in combination feeds:

It is possible that meat and bone meal contained in combination feeds for cattle produced in and before April 1996, when the MAFF requested voluntary refrainment from using ruminant-derived meat and bone meal, may have caused the occurrence of BSE. However, there is no evidence that the combination feeds given to the cases contained any meat and bone meal, indicating that the possibility is low that such meat and bone meal directly caused the occurrence of the seven cases.

(ii) Meat and bone meal contained in combination feeds as a result of cross-contamination

There are no grounds to clearly reject this hypothesis, and the possibility cannot be denied that the infection could have been caused by combination feeds for cattle that had been cross-contaminated. In this case, considering the circumstances, including the fact that there is no combination feed-producing factory that could be a common source for all seven cases, it seems likely that these cases were not infected from a common source but from different sources located both in Hokkaido and the Kanto region.

(iii) Meat and bone meal either contained in supplementary feeds or fed directly

Considering the circumstances including the fact that no evidence has been found that any meat and bone meal or any supplementary feed containing meat and bone meal was given to cattle at the farms at which the cases occurred, the possibility seems low that such meat and bone meal caused the occurrence of the seven cases.

(Supplementary feed:
Feed given to cattle by mixing them into combination feeds mainly for the supplementation of vitamins and minerals (e.g.: vitamin feed supplements, feed supplements with both vitamins and minerals).)

(iv) Meat and bone meal contained in pet foods

Although infection through this route is theoretically possible, the possibility seems to be extremely low that the cases were actually infected from such meat and bone meal.

(v) Animal fat and oil contained in milk replacer

If we assume that the seven BSE cases were infected from a single common source, contamination of their milk replacer by the pathogen would most likely explain the occurrence of these cases. In this case, the ingredient of the milk replacer that can serve as the source of infection is animal fat and oil. Since Dutch animal fat and oil was used in all seven cases, the pathogen may have been present in such animal fat and oil. However, considering the status of importation and the status of use of such products at neighboring farms, no factual evidence suggesting such a possibility has been found in the circumstances surrounding the animal fat and oil used.

(vi) Animal fat and oil used in combination feeds other than milk replacer

If the animal fat and oil used in the combination feed for calves contained as impurities proteins that were derived from the tissue of any SRM, then it seems possible that the cases were infected through ingesting such animal fat and oil. However, considering that no combination feed containing animal fat and oil was used at the farms that produced two out of the seven cases, the possibility that such animal fat and oil served as the source of infection seems even more unlikely than in the case of animal fat and oil contained in the milk replacer.

(vii) Other causes

In addition to the above, analyses and evaluations were also conducted on meat and bone meal contained in fish meal that was contained in combination feeds, as well as on animal drugs. As the results included the finding that there was no factory that could have been the common source of the fish meal used at the farms on which the cases occurred, the possibility seems to be extremely low that the aforementioned materials served as the source of infection.

(2) Statistical analysis of factors involved in the infection that were found at the farms at which the cases occurred and at other farms (a case-control study)

[Conducted by: Yasuharu Yoshida, Head of the Department of Food Policy and Evaluation, Policy Research Institute, MAFF; and Mutsuyo Kadohira, Associate Professor, International Cooperation Center for Agricultural Education, Nagoya University]

Using the epidemiological technique of a case-control study, a database was compiled containing information on feeds provided at the farms at which the cases occurred and at other neighboring farms, to compare the feeds and examine whether any particular brand(s) of feed that may be suspected to have been the cause had actually been given to the infected cattle.

The results showed that, for example, giving milk replacer had no statistical correlation with the BSE occurrence. With respect to other feeds, no factor (feed) was found to be statistically correlated with the BSE occurrence.

(3) Quantitative risk analysis based on infection route models

[Conducted by: Yasuhiro Yoshikawa, Professor, Agronomics and Life Science Research Department, Faculty of Agriculture, The University of Tokyo Graduate School]

A. Objectives

The objectives were to conduct risk evaluation, to estimate the scale of a potential future BSE occurrence, and to facilitate the epidemiological research and risk management related to such an occurrence, by preparing several scenarios representing certain combinations of risks of BSE pathogen entry through live cattle, meat and bone meal, and animal fat and oil imported from BSE countries, and the risks of exposure to the pathogen in Japan.

B. Concept of forecasting the scale of an occurrence based on the risk scenarios

(i) In the calculation of infection risk, the unit of risk is measured by the number of contaminated cattle. The infection risks of meat and bone meal and animal fat and oil are measured by the number of contaminated cattle obtained by multiplying the quantities of the product in question by a specific factor.

(ii) If a herd of cattle that includes one case animal is rendered and recycled as meat and bone meal, it is assumed that the herd includes 4 (3 to 5) infected cattle, and that the meat and bone meal derived from these cattle will amplify the extent of infection by a factor of 4 (3 to 6). Therefore, if one herd of cattle that includes one case animal is rendered and amplifies the extent of infection, the minimum and maximum number of cattle so infected will be 9 and 30, respectively.

(iii) The factor of amplification by animal fat and oil derived from one infected animal is assumed to be 8/100 (6/100 to 12/100).

(iv) Based on the aforementioned factors of amplification, the risks were calculated of BSE pathogen entry through live cattle, meat and bone meal, and animal fat and oil imported from BSE countries, considering the data on the time of importation and quantities imported, as well as the currently expected routes of infection, and risks of exposure to such a pathogen in Japan. The cases that have occurred thus far were examined and an estimate was made of the scale of any potential BSE occurrence in Japan in and after 2003.

C. Estimated scale of an occurrence

Using the risk scenarios so constructed, and assuming that live cattle and meat and bone meal, etc., imported from BSE countries have served as the sources of infection, the scale of an occurrence estimated from the quantities imported, etc., is about 10 to 20 cattle in eastern and western Japan and 8 to 13 cattle in the Kyushu area in 2003-2006. Since about 60% of these cattle will be slaughtered as healthy cattle before reaching 30 months of age (before the BSE pathogen accumulates), the numbers of BSE cattle that will be detected at abattoirs and through farm surveillance are estimated to be 7 to 9, 5 to 7 and 3 to 4 in the Kanto region, the Kyushu area and Hokkaido, respectively. However, in the Kyushu area where the percentage of beef cattle is higher, the risk of exposure to meat and bone meal and the factor of amplification through meat and bone meal are likely to be lower than the theoretically calculated levels, and the area may be less contaminated than expected.

3. Status of Overseas Investigations into Causes

Investigations into the causes have been conducted so far in Britain, the Netherlands, Denmark, and other countries where BSE has occurred. According to such investigations, direct feeding of meat and bone meal contaminated by the BSE pathogen or cross-contamination between such meat and bone meal and cattle feed are suspected as the possible sources and routes of infection, but such meat and bone meal has not been identified.

4. Presumed Sources and Routes of Infection

Based on an analysis of the source and routes of infection using the hypothesis and testing method, and on the quantitative risk evaluation using infection route models, the BSE pathogen that has occurred in Japan is considered to have been derived from Britain, directly or indirectly imported, and the possible sources and routes of infection are considered to be as follows:

(1) Sources of infection

(i) There is a possibility that cattle imported from Britain in 1982 or 1987 may have included BSE-infected cattle, some of which may have been slaughtered, processed and rendered into meat and bone meal, and some Japanese cattle may have been exposed to the pathogen potentially contained in this, and the cattle may have been recycled once again and the resulting meat and bone meal may have served as the source of infection.

(ii) The possibility cannot be denied that some Japanese cattle may have been exposed to the BSE pathogen that may have been contained in Italian meat and bone meal imported in and before 1990, and the cattle so infected may have been slaughtered, processed and rendered into meat and bone meal, and served as the source of infection.

(iii) The fact cannot be ignored that the Dutch animal fat and oil was given to the cattle in all seven cases. However, the possibility is low that the animal fat and oil contained animal proteins, and thus the possibility of it having been contaminated by the pathogen is low, if not to be excluded. From this point of view, it is difficult to associate the Dutch animal fat and oil with the cases that have occurred so far as the direct source of infection.

(2) Routes of infection

(i) Among the aforementioned sources of infection, meat and bone meal may have caused the cross-contamination of combination feeds for cattle at the manufacturing and delivery stages, as many combination feed producing factories have been found to have a production line common to cattle, pig and chicken feed.

(ii) In Britain, many BSE cases have been identified even in cattle called "BAB (born after the ban)," which are cattle born in and after 1988, when the feeding of meat and bone meal to cattle was banned, and those cattle are presumed to have been infected mainly due to cross-contamination. It thus seems likely that the infection in Japan may also have been caused by cross-contamination.

(iii) As for animal fat and oil, since it was added to the milk replacer as an ingredient, it may have directly caused the infection if it contained the pathogen. However, as mentioned above, it is difficult to associate this with the cases as a direct route of infection.

5. Risk Management Based on the Results of the Investigations

(1) Blocking infection routes

Since the BSE occurrence in September 2001, various measures have been taken to control the risks associated with the introduction of and exposure to meat and bone meal, including regulations governing its use in feed, as well as to block new infection (Attachment 2).

(2) Proposals for prevention of the future spread of BSE

A review follows of the measures currently being taken for the prevention of the spread of BSE. Firstly, the importation, manufacture and shipment of meat and bone meal, etc., as feed or fertilizer were totally suspended for a period and, from a legislative point of view, the Law Concerning Safety Assurance and Quality Improvement of Feed (hereinafter referred to as "Feed Safety Law") (October 15, 2001) and the Law on Special Measures Against Bovine Spongiform Encephalopathy (hereinafter referred to as "BSE Special Measures Law") (effective from July 4, 2002) banned the feeding of meat and bone meal, etc., to ruminants. Secondly, screening of all cattle at abattoirs started from October 18, 2001, and, in addition, it became mandatory to incinerate the specified risk materials (SRM) from cattle processed at abattoirs. Other measures that have been taken include the restriction of animal fat and oil products that can be used in cattle feed to those that are derived from fat collected from meat for human consumption and contain less than 0.02% of insoluble impurities (Notification from the Director of the Agricultural Production Bureau dated December 27, 2001; an amendment to the Ministerial Ordinance dated August 2, 2002; and the Notification from the Director of the Agricultural Production Bureau dated March 19, 2003).

It seems that the various sources and routes of infection contemplated in the present epidemiological investigation have been completely blocked by these measures.

The essential points for the prevention of the spread of BSE from an epidemiological point of view can be summarized as follows. It is advisable that these points are referred to in the proper operation of the aforementioned measures.

(i) The analysis using the risk scenarios indicates the possible risks of the introduction and exposure involved in the importation of live cattle from Britain, the importation of animal fat and oil from the EU, and the importation of meat and bone meal from the EU, as well as the possible amplification of contamination by the rendering of cattle in Japan.

(ii) The fact that the dates of birth of the seven BSE cases are very close and that the distribution of all the cases was confined to eastern Japan suggest that the source of infection has not been widespread.

(iii) The risk scenarios presume that more than one route of infection existed. The hypothesis and testing method points out the possibility that different sources of infection may have existed in Hokkaido and the Kanto region.

(iv) Assuming that BSE-contaminated cattle that existed up to 2001 were rendered, there may occur in the future BSE-positive cases that are infected from a source or sources different from that or those in the BSE cases born in 1995 and 1996.

(v) The factors, such as animal drugs, that have been epidemiologically considered to have a low possibility of having served as a source of infection may, if seen from a different perspective, cause a major outbreak once they become a source of infection.

(vi) The present investigation has been conducted by making many assumptions.

Considering that the occurrence of "Born After the Real Ban (BARB)" cases in Britain suggests the possibility that imported feed materials may also have been contaminated, new assumptions may be made in the future.

(vii) Each new BSE case potentially detected in the future must be checked for consistency with the hypotheses proposed in the present report. It is necessary to continue the epidemiological investigation through surveillance in future.

(viii) Although epidemiological methods have been used for a long time in medical fields, there have not been many cases in which a comprehensive, descriptive epidemiological method or a method of quantitative risk evaluation was employed in the analysis of the source or routes of infection for a livestock disease. Such veterinary epidemiological methods should be actively used in the future investigation of measures against livestock infectious disease.

Conclusion

In the current globalized society, the importance of measures against newly emerging and re-emerging infectious diseases has been recognized. Among such diseases, BSE is a typical newly emerging infectious disease born out of the modern livestock industry. BSE has shown a worldwide geographic pattern of occurrence, spreading from the European countries to Japan, Israel and Canada. The occurrences of BSE in these countries have been detected through abattoir screenings or surveillance, while the status of BSE contamination in other countries where such measures have not been taken is still unknown. The fact that British meat and bone meal suspected of BSE contamination that had been produced by the time of the massive occurrence of BSE cases in Britain had been exported in large quantities to many other countries in the world suggests the possibility that BSE contamination may have spread worldwide. Although Japan has now taken prudent measures to prevent the re-entry of BSE, the possibility must continue to be considered that the re-entry of BSE into Japan may occur from overseas through any of the various routes indicated in this report. We sincerely hope that the present report will be made the best use of in measures to be taken in the future for the prevention of the re-entry of BSE.

Attachment 1

Summary of BSE-Infected Cattle Discovered So Far

	Date of occur-rence (Note 1)	Location of farm (style of management)	Breed	Date of birth (age by month)	Number of cattle kept at time of occurrence (number of suspected cases)
1st case	Sept. 10, 2001	Shiroi-shi, Chiba Prefecture (dairy farm)	Holstein	Mar. 26, 1996 (65 months old)	46 (44)
		(Introduced from) Saroma-cho, Hokkaido (dairy farm)			Farm had already been closed at the time of occurrence.
2nd case	Nov. 21, 2001	Sarufutsu-mura, Hokkaido (dairy farm)	Holstein	Apr. 4, 1996 (67 months old)	82 (62)
3rd case	Dec. 2, 2001	Miyagi-mura, Gunma Prefecture (dairy farm)	Holstein	Mar. 26, 1996 (68 months old)	68 (56)
4th case	May 13, 2002	Onbetsu-cho, Hokkaido (dairy farm)	Holstein	Mar. 23, 1996 (73 months old)	56 (44)
5th case	Aug. 23, 2002	Isehara-shi, Kanagawa Prefecture (dairy farm)	Holstein	Dec. 5, 1995 (80 months old)	47 (37)
6th case	Jan. 20, 2003	Kokawa-cho, Wakayama Prefecture (dairy farm)	Holstein	Feb. 10, 1996 (83 months old)	51 (0)
		(Introduced from) Shibecha-cho, Hokkaido (dairy farm)			98 (27)
7th case	Jan. 23, 2003	Abashiri-shi, Hokkaido (dairy farm)	Holstein	Mar. 28, 1996 (81 months old)	131 (0)
		(Introduced from) Yubetsu-cho, Hokkaido (dairy farm)			59 (7)

Note 1: The date on which the case was confirmed to be positive as a result of BSE testing for the 1st case, and the date of definitive diagnosis for the other cases.

Note 2: In addition to the above, one positively screened animal (an animal raised for beef (Japanese Black) of 241 months of age) that was processed at an abattoir in Kanagawa Prefecture on February 5, 2003, was not determined to be negative for BSE at the meetings of the "Expert Committee for the Testing of Bovine Spongiform Encephalopathy (BSE)" held on February 8 and March 27.

Attachment 2

Causes of the BSE Outbreak in Japan and Risk Management

The following could conceivably have caused the BSE outbreak in Japan. Risk management is now being implemented to prevent further infection.

(1) Infection due to domestic recycling of infected live cattle imported from BSE-affected countries (i.e. contaminated cattle are processed as MBM after slaughter, and this cross-contaminates cattle feed).

(2) Direct infection due to consumption of cattle feed that has been cross-contaminated by contaminated MBM imported from BSE-affected countries, as well as infection due to domestic recycling.

(3) Direct infection due to consumption of cattle feed made from contaminated animal fat imported from BSE-affected countries, as well as infection due to domestic recycling.

	Cause of Outbreak	Risk Management
Introduction Risk	Imported live cattle	• Ban on import of live cattle from BSE-affected countries (Animal Health Conditions) • For cattle imported from BSE-affected countries and reared in Japan, request to ascertain movements, conduct BSE testing on fallen and slaughtered stock, and ascertain results (Notification) • BSE testing and removal of SRM from all animals at slaughterhouses, block on distribution of BSE-positive cattle for food or feed (BSE Special Measures Law) • BSE testing of fallen stock aged 24 months or more (BSE Special Measures Law)
	MBM	• Provisions including a ban on use of feed containing cattle MBM (BSE Special Measures Law) • Provisions to prevent feed for cattle, sheep and others from containing mammalian-derived or poultry-derived protein (except milk, milk products, etc.) (Feed Safety Law, Ministerial Ordinance) • Ban on feeding mammalian-derived or poultry-derived protein to cattle, sheep and others (Feed Safety Law, Ministerial Ordinance) • Chicken meal and pig- or horse-derived blood meal may only be used in chicken, pig and fish feed if it has been confirmed by the Minister of MAFF to have undergone separate manufacturing processes that prevent admixture of cattle MBM (Feed Safety Law, Ministerial Ordinance) • Suspension of manufacture and factory shipments of MBM for feed and fertilizer, and of feed and fertilizer that contains MBM (Notification)
	Animal fat	• Animal fat used in feed limited to that with a maximum 0.15% content of insoluble impurities (weight conversion), and animal fat used in milk replacer for cattle limited to that derived from fats extracted from meat and with a maximum insoluble impurity content of 0.02% (Feed Safety Law, Ministerial Ordinance) • Tallow used in the manufacture of cattle feed limited to that derived from fats extracted from meat and with a maximum insoluble impurity content of 0.02% (Notification)
Exposure Risk	Cross-contamination at compound feed factory	• Imposition of guidelines to prevent admixture of ruminant derived proteins in ruminant feed (Notification) • Provisions to the effect that feed for cattle, sheep and others must be manufactured in processes completely separated from manufacturing processes that use mammalian-derived, poultry-derived, or seafood-derived protein (Feed Safety Law, Ministerial Ordinance, enforced from April 1st, 2005)
	Feeding of contaminated feed	• Ban on feeding mammalian-derived, poultry-derived, or seafood-derived protein to cattle, sheep and others (Feed Safety Law, Ministerial Ordinance) • Mandatory storage of mammalian-derived, poultry-derived, or seafood-derived protein, and feed containing these, to prevent admixture with feed for cattle, sheep and others (Feed Safety Law, Ministerial Ordinance) • Mandatory indication on mammalian-derived, poultry-derived, or seafood-derived protein, and feed containing these, of the content of aforementioned provisions, as "Cautions when Using and Storing" (Feed Safety Law, Ministerial Ordinance)

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Nevertheless, two questions remained unanswered. Namely, what was the source of infection of Japanese cases of BSE? And what route did the infection take? The Ministry of Agriculture, Forestry and Fisheries (MAFF) has conducted investigations starting from farms in which the 7 cases so far have occurred, and another survey that traced meat-and-bone meal (MBM) and other products imported from countries with known incidence of BSE. The results of these surveys have been published in the form of two reports. However, the these investigations were conducted by the administration, and did not evaluate the issue from an epidemiological aspect (an important scientific means of investigating sources of infection).

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Kazuya Yamanouchi, Senior Researcher, Nippon Institute for Biological Science / Emeritus Professor, University of Tokyo

Chapter 1 Cattle Farming Formats in Japan and an Outline of Previous Investigations on Infection Sources and Infection Routes

1 Cattle farming formats in Japan

The formats for farming cattle in Japan can be divided into two types, i.e. dairy farming for the production of raw milk as a raw material for milk and dairy products, and beef cattle farming for the production of beef. As of February 1st, 2003, some 1.72 million dairy cows were being farmed on about 30,000 dairy farms, and 2.8 million beef cattle on around 98,000 beef cattle farms.

The majority of dairy cattle are Holsteins. About 60% of beef cattle are Wagyu (Japanese cattle) and other exclusively beef-designated breeds, with the remaining 40% taken up by Holstein bulls and Holstein-Wagyu crossbreeds.

The principal farming formats for these are shown in Appendix 1 (Life Cycles of Cattle).

1.1 Dairy cattle farming format

After birth, female dairy cattle are reared on dairy farms as candidates for fresh milk production (mother cows). Male dairy cattle are sold to calf fattening farms as beef calves shortly after birth. Since, in the case of dairy cattle, the cow's milk is sold as a product (fresh milk), calves are separated from their mothers and reared on milk replacer instead of cow's milk.

The milk replacer is mainly skimmed milk powder with added nutrients such as minerals and fats. The powder is dissolved in hot water and then fed to the calves. Milk replacer is generally fed to calves for about 1 month after birth, after which feeding switches to calf starter fed in powdered form. At the same time, the calves are fed with roughage (hay and pasture grass). Calf starter is fed up to around 3-6 months of age. In some cases, however, compound feed is given from around 1 month of age; the rearing method differs somewhat from farm to farm. After 3 months of age, feed generally takes the form of compound feed combined with roughage.

Female dairy cattle are mated at around 18 months of age (by artificial insemination or embryo transfer). They deliver their first calf at around 27 months, when they first produce raw milk. Milking for raw milk production continues for about 1 year after parturition. In the meantime, the cows are mated for a second time, and milking continues again after the second calf is delivered. In this way, parturition and milking are repeated in cycles (with 4 calves produced on average). However, when milk yield decreases and the feed efficiency declines, their role as milking cattle is finished and they are slaughtered for beef.

1.2 Beef cattle farming format

Beef cattle are broadly divided into breeding stock (cows) for calf production and fattening cattle for meat production.

Breeding cattle start to produce calves from around 2 years of age. After delivering up to around 7 calves, they are shipped for beef production. Of the calves produced, superior females are reared for breeding, while males and females with less potential for production become fattening cattle for beef.

Fattening cattle are subjected to specialized rearing management from around 10 months of age. They are shipped at around 29 months of age and 700kg in weight. There is a big difference in the feeding methods used for breeding and fattening cattle. For breeding cattle, roughage is the main ingredient, as excessive fattening can cause reproductive disorders. For fattening cattle, conversely, the main ingredient of feed is high-energy concentrate (compound feed) for greater efficiency of meat production. By definition, it follows that most of the cattle seen grazing on pastures are breeding cattle. In the case of beef cattle, cow's milk is not processed as a commercial product (raw milk). Therefore, calves are usually reared together with their mothers and are nursed on cow's milk. Milk replacer or other substitutes are rarely used.

1.3 Supply and demand for cattle feed

Herbivores by nature, cattle are physiologically able to grow, breed, and produce milk merely by grazing on pastures. To accelerate growth, increase milk yield, and improve meat quality, however, they are given compound feed containing maize, sorghum, oats, soybean oil cake, and other fodder cereals as main ingredients. Fodder cereals that are rich in nutrients per unit of weight are generally known as "concentrate", and compound feed comes under this heading. Pasture grass, on the other hand, is defined under the general heading of "roughage". This includes fresh grass, dried grass (hay), rice straw, and silage (fermented feed). The average dairy farm in Japan feeds adult Holstein cattle with about 10kg of roughage (hay) and about 8kg of concentrate (compound feed) each day.

Feed self-sufficiency in FY2001 was 77% for roughage and 10% for concentrate. There is an overwhelming dependence on imports for the latter. This is particularly so in the case of maize, which accounts for about 5% of compound feed ingredients and is nearly all imported.

Compound feed for cattle is prepared from carbohydrates, protein, fats, minerals and others in accordance with the feed requirement. The main ingredients used are maize, sorghum, oats, wheat bran, soybean oil cake, rape-seed oil cake and other plant-derived ingredients. Vitamins, minerals and other supplements are often given in addition to compound feed and roughage.

Many different types of compound feed are manufactured and marketed, depending on the growth stage and purpose. These include the milk replacer given to nursing calves and calf starter fed in the latter stages of nursing, as well as compound feed specially prepared for female dairy cattle and beef cattle, respectively.

1.4 Distribution of meat-and-bone meal (MBM)

Sources of protein are indispensable as feed ingredients. They include plant-derived protein such as soybean oil cake and rape-seed oil cake, and animal-derived protein such as MBM and fish meal.

MBM is made by rendering the offals (meat scraps, bone, organ remains, etc.) produced when processing livestock at slaughterhouses. These offals are dried and powdered after separating oils and fats. Until their use was restricted owing to the outbreak of BSE, these were widely used as ingredients for feed, fertilizer and pet food. As for the supply and demand in FY2000, approximately 400,000 tons were produced by 121 rendering plants in Japan, while another 170,000 tons or so were imported. Of the total, 420,000 tons were used as compound feed ingredients for swine and poultry (see Appendix 2).

Unlike pigs and chickens, cattle do not need animal protein (they use micro-organisms in the rumen as protein). As such, MBM has not traditionally been used so much as an ingredient for compound feed. Until its use in cattle feed was

banned under administrative guidance on April 16th, 1996, the annual usage was 247 tons in FY1995, 131 tons in FY1994, and 124 tons in FY1993. This was about 0.05% of the use of MBM as an ingredient for compound feed (see Appendix 3).
However, in on-site inspections of whole herds at all cattle-rearing farms after the BSE outbreak, some farms were found to be feeding MBM and other prohibited substances, unaware of the administrative guidance on restricted use. It was confirmed that about 5,000 cattle in these farms were being fed with MBM and others. The farms are thought to have used MBM and others as supplementary feed to increase milk yield, etc., in view of their protein and mineral content. As the background to the restriction on use of MBM, the government's Inquiry Committee on BSE Issues states that "The fact that the problem was merely handled by administrative guidance via notification from a division manager, despite the WHO recommendation for a ban on MBM in April 1996, can only be described as a grave error of policy, even considering that imports of MBM from the UK had been halted and there was hardly any use of MBM in cattle feed." This has been one of the major lessons learned in the course of the BSE problem.

2 Outline of previous surveys

2.1 Content of surveys

Since BSE was first confirmed in September 2001, two investigations have been conducted on the 7 cases confirmed so far. The first was a "downstream" investigation tracing feed and others that infected cattle may have consumed, starting from the farms in which the 7 cases occurred. The second was an "upstream" investigation tracing distribution routes and other means whereby MBM and others imported from BSE-affected countries were distributed to producing farms.

2.2 Outline of the investigation results

2.2.1 Investigation starting from farms with confirmed cases of BSE

2.2.1.1 Outline of infected cattle

So far, 7 cases of BSE infected cattle have been confirmed in Japan. These are summarized in Table 1-1 below. All 7 cattle were Holsteins, born between December 1995 and April 1996. The age at the time of outbreak was between 5 years 5 months and 6 years 11 months. Of the 7 cattle, 5 were bred in Hokkaido, and 1 each in Gumma and Kanagawa Prefectures. Apart from these, a Japanese Black (beef cattle) aged 2 years 1 month, which was processed at a slaughterhouse in Kanagawa Prefecture on February 5th, 2003, and proved positive in screening tests, has not been proven negative in meetings of Expert Panel on BSE Testing held on February 8th and March 27th.

Table 1-1 Summary of BSE infected cattle confirmed so far

	Date of outbreak	Location of farm	Date of birth (age)	Herd size at time of outbreak (of which, suspected animals)	Remarks
1	10/09/2001	Shirai City, Chiba Prefecture (Breeding farm) Saroma-cho, Hokkaido	26/03/1996 (5yrs 5mths)	46 (44) (Breeding farm) No longer in business at time of outbreak	Recalled due to astasia

2	21/11/2001	Sarufutsu Village, Hokkaido	04/04/1996 (5yrs 7mths)	82 (62)	No abnormality found in ante-mortem test at time of shipment
3	02/11/2001	Miyagi Village, Gumma Prefecture	26/03/1996 (5yrs 8mths)	68 (56)	No abnormality found in ante-mortem test at time of shipment
4	13/05/2002	Onbetsu Village, Hokkaido	23/03/1996 (6yrs 1mth)	56 (44)	Recalled due to myorrhexis (injury) to right forelimb
5	23/08/2002	Isegahara City, Kanagawa Prefecture	05/12/1995 (6yrs 1mth)	47 (37)	Recalled due to dislocated hip joint
6	20/01/2003	Kokawa-cho, Wakayama Prefecture (Breeding farm) Shibecha-cho, Hokkaido	10/02/1996 (6yrs 11mths)	51 (0) (Breeding farm) 98 (27)	Shipped as healthy stock, but collapsed and had difficulty in standing while tethered, so slaughtered in sick bay
7	23/01/2003	Abashiri City, Hokkaido (Breeding farm) Yubetsu-cho, Hokkaido	28/03/1996 (6yrs 9mths)	131 (0) (Breeding farm) 59 (7)	No abnormality found in ante-mortem test at time of shipment

(Geographical relationship between farms with confirmed BSE cases)

2.2.1.2 Testing of suspected animals

Of cattle that had some connection with infected animals (such as cattle in the same herd), suspected animals were identified and slaughtered. Suspected animals were defined as (1) cattle that had been in the same herd as a confirmed animal up to one year of age, and which may have been given the same feed as a confirmed animal; (2) cattle that were born in the twelve months before and after the birth of a confirmed animal in the same farm (herd) as a confirmed animal, and which may have been given the same feed as a confirmed animal; and (3) calves born of a confirmed animal in the two years before manifestation of BSE in the confirmed animal, or after manifestation. After slaughter, these 361 suspected animals were subjected to BSE testing, but all proved negative.

New standards on the criteria of suspected animals were stipulated at a General Meeting of the Office International des Epizooties (OIE) in May 2003. The criteria of suspected animals as defined in these standards were consequently applied in Japan from June 25th, 2003 (see Appendix 4).

2.2.1.3 Feeding investigation

On-site investigations were conducted by Animal Quarantine Officers from Prefectural Livestock Hygiene Service Centers with jurisdiction over the affected farms. As well as interviewing farm personnel, the Officers also investigated sales receipts and other documentation of feed suppliers. The results of these investigations were collated to provide data on the feed used by these farms while they were rearing infected cattle, as shown in Table 1-2 below. As a result, no evidence of feeding with MBM (regarded as the source of BSE infection) was found.

Table 1-2 Identified types of feed supplied

	Compound feed	Supplementary feed	Simple substance feed	Roughage
Case 1 (Breeding farm) (Shipping farm)	8 types 2	5 types 2	5 types 3	1 type 2
Case 2	9	18	1	–
Case 3	9	1	5	1
Case 4	6	5	4	–
Case 5	4	10	3	6
Case 6 (Breeding farm) (Shipping farm)	4 3	1 4	3 6	1 5
Case 7 (Breeding farm) (Shipping farm)	5 8	3 17	– 10	– 13

Note: Supplementary feed includes silage additives. Roughage does not include self-produced roughage.

2.2.1.3.1 Compound feed

Feed inspectors of the Fertilizer and Feed Inspection Station (an Independent Administrative Institution) conducted on-site inspections of related compound feed factories, including those producing milk replacer and calf starter. As a result, it was confirmed that none of them used MBM as an ingredient in compound feed. However, 7 of these factories also produce pig and chicken feed including MBM as an ingredient. Since these share the same production lines as cattle feed, the possibility of contamination with MBM in the manufacturing or shipping stages could not be completely ruled out.

Nearly all related feed factories, moreover, used animal-derived feed ingredients such as fish meal and domestically produced animal fat. The origins, compositions and other aspects of these were investigated, but all of them were proved unlikely as sources of BSE infection.

Table 1-3 Related compound feed factories

	Case 1 Hokkaido	Case 1 Chiba	Case 2	Case 3	Case 4	Case 5	Case 6 Hokkaido	Case 6 Wakayama	Case 7
Scientific Feed Laboratory Ltd. – Takasaki Factory	○		○	○	○	○	○		○
Hokuren Kumiai Shiryo – Kushiro-Nishi Minato Factory *	○		○		○		○		○
– Kitami Factory *	○								○
Nihon Nosan Kogyo – Otaru Factory	○								
Zenrakuren – Kashima Feed Factory		○							
– Kushiro Feed Factory			○				○		○
Mercian – Tomakomai Factory			○						
Kanto Kumiai Chemical Industry – Akagi Factory *				○					
Meiji Shiryo – Kashima Factory *						○			
Zenrakuren – Kansai Feed Factory								○	
Nishi Nihon Shiryo								○	
Kushiro Shiryo – Kushiro Factory *									○
Chubu Shiryo – Hokkaido Factory *									○

Note: In factories marked * , the possibility of contamination with MBM in the manufacturing or shipping stages could not be completely ruled out.

2.2.1.3.2 Milk replacer, calf starter

Common to all 7 cases of infected cattle is that they used milk replacer produced in the same factory (the Takasaki Factory of Scientific Feed Laboratory Ltd.). On conducting detailed investigations there, we found that MBM was not used as an ingredient in this milk replacer. However, powdered fat (animal fat mixed with casein or other lactoproteins and pulverized) imported from the Netherlands, a country with incidence of BSE, were used.

Therefore, the MAFF sent staff to the Netherlands to investigate the origin of this animal fat, the manufacturing processes, and so on. They were, however, unable to find any evidence of contamination with bovine protein that could be the source of BSE infection.

As for calf starter, there was no producing factory common to all 7 cases, of which Cases 4 and 5 had not been fed with calf starter at all. All of the animal fat used was produced in Japan.

Table 1-4 Feeding of milk replacer and calf starter, and origin of animal fat

	Brand name	Producing factory	Origin of fats	Case 1	2	3	4	5	6	7
Milk replacer	Miru Food A Super	Scientific Feed Laboratory Ltd., Takasaki Factory	Japan, USA, Netherlands	○	○		○		○	○
	Pure Milk H	"	"		○					
	Pure Milk	"	"			○		○		
	Miru Food B Flake	Hokuren Kumiai Shiryo, Kushiro-Nishi Minato Factory	Japan	○						

Calf starter	Miru Food B Green	"	"	○ ○
	Miru Food B	"	"	○
	Kumiai X Morlet	Kanto Kumiai Chemical Industry, Akagi Factory	"	○

2.2.1.3.3 Other feed

We investigated the supplementary feed, single substance feed and purchased roughage that were used by the farms with confirmed cases. In all cases, these proved unlikely to have been sources of infection. We also investigated the fish meal content of compound feed. Although mammalian protein was detected in fish meal at some fish meal factories, this was thought to have been present in food scraps used as an ingredient in the meal.

2.2.1.4 Other investigations

We also surveyed fertilizers and pet food purchased and used on the farms, as well as veterinary pharmaceuticals administered to the infected cattle, to ascertain the possibility that they had mistakenly eaten or been fed fertilizer or pet food other than cattle feed, or the possibility of infection from veterinary pharmaceuticals. As a result, all of these proved unlikely to have been sources of infection.

2.2.2 Investigations starting from imported MBM, etc.

2.2.2.1 Imported MBM

2.2.2.1.1 United Kingdom

According to Japanese trade statistics, there have been no purchases of MBM from the UK since 1980. EU statistics, on the other hand, record exports of 333 tons to Japan between 1990 and 1996. The MAFF therefore sent officers to investigate this. Their findings were that exports to Japan totaled 227.6 tons, and were highly likely to have been non-ruminant meal. The remaining 105.4 tons had been shipped to other countries.

Between 1995 and 2000, more than 9,000 tons of bone meal and others were imported from the UK. On investigating these, it was confirmed that they consisted of edible bone meal (134 tons) processed at high temperature and high pressure (moist heat sterilization at 136°C, 40psi (about 3 bar) for at least 70 minutes), and edible bone (9,063 tons), nearly all of which was derived from pigs.

2.2.2.1.2 Denmark

A total of 30,533 tons of MBM were imported from Denmark between 1999 and 2001. The MAFF sent officers to investigate this, and their findings showed that imports of MBM from Denmark started in December 1999. Both of the factories that exported MBM to Japan (2 companies, 2 factories) used domestically produced raw materials, which were treated at high heat and high pressure according to treatment standards based on EC directive 96/449/EC (133°C, 3 bar, 20 minutes).

2.2.2.1.3 Italy

A total of 55,930 tons of MBM have been imported from Italy since 1987. Of this total, 55,068 tons have been imported since 1998.

The MAFF sent officers to Italy to investigate this. Their findings showed that all exports of MBM to Japan were made through company A, which had installed heating and pressurizing equipment on June 1st, 1998. Since then, the company had been manufacturing MBM according to treatment standards based on EC directive 96/449/EC (133°C, 3 bar, 20 minutes).

It also became clear, however, that the heating equipment used for MBM exported to Japan before June 1st, 1998, did not have a pressurizing mechanism, and therefore that heat treatment at 3 bar, needed to effectively inactivate the BSE agent, was not carried out. Moreover, the structure of the equipment was such that steam did not come into contact with the MBM. Also, the possibility could not be ruled out that bovine specified risk material (SRM) was included among the raw materials of MBM imported between 1996 and June 1st, 1998. It also became clear that the factory that exported MBM to Japan may have used raw materials imported from BSE-affected countries (Ireland, France, etc.). We requested additional information from the Italian government authorities, and were supplied with relevant data. However, since the period in question goes back 6 or 7 years, not enough information was obtained to rule out these possibilities.

Meanwhile, on conducting on-site inspections of Japanese importers, we were not able to ascertain the content or purpose of use of MBM imported up to 1990, as there are no surviving documents or other data from that time. However, it was confirmed that the 20 tons of MBM imported in 1993 originated from poultry. We also discovered that the 21 tons imported in 1995, the 60 tons imported in 1997 and the 420 tons imported between January and June 1998 were used as ingredients for pet food or fish food. As for the 105 tons imported by Mitsui & Co. in November 1996, it was judged highly likely, from interviews with related personnel, etc., that these were used as ingredients in chicken feed. This could not, however, be accurately confirmed from documents or other data.

2.2.2.1.4 Other European countries

According to trade statistics, 47 tons of German MBM were imported in 1992. Our investigation proved that these were all used as ingredients in fertilizer. Records also show that 38 tons of Russian MBM were imported in 1993. However, this could not be confirmed as there is no record of testing by the MAFF's Animal Quarantine Service and no surviving data in the Customs & Tariff Bureau of the Ministry of Finance. The statistics also show that 105 tons of Austrian MBM were imported in 1996, 99 tons in 1997, and 204 tons in 1999. However, there are no test records concerning these at the Animal Quarantine Service, and it was conjectured that "Austria" was a mistake for "Australia". Besides these, a total of 835 tons of bone meal were imported from Ireland between 1991 and 1998. In interviews with importers, however, it was shown that these were used, among others, as calcium ingredients for health foods, and there was no possibility that they were used as feed ingredients.

2.2.2.1.5 Asia

Although there have been no reports of BSE outbreaks in other Asian countries, the possibility cannot be ruled out that MBM produced in BSE-affected countries has been imported to Japan via Asia. Therefore, the MAFF sent officers to Hong Kong, Thailand, Indonesia, the Philippines, South Korea, Taiwan and China to investigate their respective imports and exports of MBM.
As a result, it became clear that imports of MBM from BSE-affected countries had been prohibited by all of these countries in around 1996, or earlier, and that imported MBM was mainly used as a domestic feed ingredient for pigs and poultry. It also became clear that there would be little merit in re-exporting MBM

to Japan once it had been imported into those countries, in view of the transportation costs, customs tariffs, commissions, and so on. Nevertheless, Hong Kong is known to function as a mid-way base for trade, and exports about 6,000 tons to Japan every year on average. Therefore, we particularly investigated the origin, purpose and other aspects of imported MBM since March 1996 (when Japan banned imports of MBM from the UK). As a result, it was confirmed that 218 lots (8,337 tons) were imported between March 1996 and December 1997, of which 187 lots (7,516 tons) were pig-derived and 23 lots (591 tons) were sold to fertilizer companies. Details of the remaining 8 lots (230 tons), unloaded at Osaka, Komatsushima, Kobe and Moji, were unknown. It was also confirmed that, among imports since 1998, there was 1 lot of mixed-animal MBM, but that it was sold to a fertilizer company. The remaining 155 lots were all pig-derived, and all but one of these were meat meal.

2.2.2.1.6 Canada

In Canada, the first case of BSE infection in domestically-bred cattle was confirmed in May this year (2003). Hundreds or thousands of tons of MBM are imported from Canada every year, and the possibility cannot be ruled out that the BSE agent already existed in Canada at around the time the infected animal, said to be 6 years old, was born (1997). Therefore, we investigated the situation of use and other aspects of Canadian MBM imported into Japan. As a result, it was confirmed that a total of 9,103 tons of MBM and meat meal were imported in the ten years between 1992 and 2001, and that these had already been shipped to locations west of Ibaraki Prefecture. In terms of the import situation at the time of birth of BSE infected cattle in Japan, in particular, it was confirmed that 688 tons were imported in 1995, 433 tons in 1996, 499 tons in 1997, and 1,880 tons in 1998, and that these were shipped to the Kanto, Tokai and Kyushu regions.

2.2.2.2 Imports of live cattle

From BSE-affected countries, 33 dairy cattle have been imported from the UK and 16 from Germany. Besides these, between 100 and 800 cattle (both dairy and beef) are imported every year from Canada, where the first incidence of BSE in domestically-bred cattle was confirmed in May this year. The results of a follow-up investigation on these imported live cattle are shown below.

2.2.2.2.1 United Kingdom

(a) Dairy cattle imported in 1982 (1 male, 4 female)

1) Month of import: May 1982
2) Destination: Kanto region
3) Dates of birth: November 1979 – November 1980
4) State of recall, etc.

Year of recall	Reason for recall
1984	2 cattle recalled (displacement of abomasum, external injury)
1985	1 cattle recalled (reproductive disorder)
1987	1 cattle recalled (postnatal astasia)
1989	1 cattle recalled (decreased milk yield)

(b) Dairy cattle imported in 1987 (9 females)

1) Month of import: October 1987
2) Destination: Kanto region
3) Dates of birth: October 1985 – March 1986

4) State of recall, etc.

Year of recall / culling	Reason for recall / culling
1989	1 cattle recalled (reproductive disorder)
1992	3 cattle recalled (deceased milk yield, suspicion of endocarditis)
1993	3 cattle recalled (reproductive disorder, diminished milk yield, mastitis)
1995	1 cattle recalled (mastitis and postnatal sickness)
1996	1 cattle culled (destroyed and incinerated after BSE testing (negative))

(c) Dairy cattle imported in 1988 (19 females)
1) Month of import: April 1988
2) Destination: Kyushu region
3) Dates of birth: September 1985 – September 1986
4) State of recall, etc.

Year of recall / death	Reason for recall / death
1989	3 cattle recalled (reproductive disorders, arthritis)
1990	1 cattle recalled (mastitis)
1991	1 cattle recalled (displacement of abomasum) 1 cattle died (acute pneumonia)
1992	5 cattle recalled (gastroenteritis, mastitis, reproductive disorders, low milk yield) 1 cattle died (ketosis)
1993	5 cattle recalled (reproductive disorders, mastitis)
1995	1 cattle recalled (mastitis)
1996	1 cattle recalled (displacement of abomasum)

2.2.2.2.2 Dairy cattle imported from Germany in 1993 (16 females)
1) Month of import: September 1993
2) Destination: Hokkaido
3) Dates of birth: April 1991 – February 1992 (1 born in the Animal Quarantine service, MAFF, in September 1993)
4) State of recall, etc. (3 cattle still alive as of September 2003)

Year of recall / death	Reason for recall / death
1997	1 cattle died (fatal fall)
1998	1 cattle recalled (mastitis)
1999	1 cattle died (gangrenous mastitis)
2001	1 cattle recalled (old age) 1 cattle died (minor piroplasmosis)
2002	7 cattle recalled (slaughtered and tested for BSE (negative))
2003	1 cattle recalled (slaughtered and tested for BSE (negative))

2.2.2.2.3 Canada

Between 100 and 800 live cattle (including both dairy and beef breeds) are imported from Canada every year. A total of 5,210 cattle have been imported from Canada since 1989, and have been shipped to destinations in 40 prefectures around the country. Of these, 754 are confirmed to be still alive, 2,050 have been shipped to slaughterhouses, 1,420 have died or been recalled, and the circumstances of the remaining 986 are unknown. As for imports of Canadian cattle born at the same time as the BSE-infected cattle, 278 were imported in 1995-1996 (93 still alive, 115 shipped to slaughterhouses, 55 died or recalled, 15 unknown) and 218 in 1997-1998 (153 still alive, 36 shipped to slaughterhouses, 26 died or recalled, 1 unknown).

2.3 Investigation results suggesting possible sources of infection

From detailed surveys of the 7 cases known so far, the possibility of sources or routes of BSE infection in Japan cannot be ruled out in the following cases.

2.3.1 Insufficiently heated Italian MBM

We discovered that MBM imported from Italy prior to June 1998 had not been sufficiently treated according to heat treatment conditions indicated in OIE standards (133°C, 3 bar, 20 minutes). Moreover, there was a possibility that MBM imported from Ireland, France and other BSE-affected countries had been used an ingredient in some of the MBM. Accordingly, we cannot rule out the possibility that this was the source of the BSE infection in Japan.

Bearing in mind that we could not rule out cross-contamination of MBM at the manufacturing stage in the 7 factories involved variously in the 7 cases, we should also consider the possibility of an infection route whereby Italian MBM was distributed to factories related to the 7 cases by some route or other (even though we could not confirm whether or not it was used in feed factories related to the 7 confirmed cases).

2.3.2 Milk replacer containing Dutch animal fat

The risk of BSE infection is said to be high in the 1st and 2nd years of a cow's life. Milk replacer is given for about 1 month after birth, and all 7 confirmed cases were fed with milk replacer produced in the same factory. Animal fat from the Netherlands, a BSE-affected country, was used as an ingredient in this milk replacer. Although animal fat itself is not thought to carry BSE infectivity, it is theoretically possible that it could become a source of infection if contaminated with brain, spinal cord and other proteins containing the BSE agent in the manufacturing process.

However, no evidence of protein contamination of animal feeds was found during field investigations in the Netherlands.

3 Overseas investigations on the source of infection

3.1 United Kingdom

Following the outbreak of BSE, cross-sectoral epidemiological research was carried out by the UK's Central Veterinary Laboratory (CVL) from the end of 1986. In 1988, the CVL published a report suggesting that oral infection through the consumption of MBM was the main infection route. Based on this, all feeding of MBM to ruminants was banned. As a result, BSE incidence decreased dramatically after peaking in 1993 (i.e. following the average incubation period of 5 years). As for other infection routes, meanwhile, the involvement of milk was ruled out by extensive cohort surveys. Maternal infection was also studied using 600 calves in an epidemiological experiment lasting 7 years. As a result, it was suggested that there was a maximum 10% chance of maternal infection. This figure was later amended to 0.5% or less.

As for the origin of the BSE agent, the theory that it originated in scrapie was proposed by epidemiological researchers.This theory was based on the chronological coincidence of the estimated time of BSE appearance with the time of change in the rendering processes and increased incidence of scrapie. This theory has become widely accepted. However, the UK government's BSE Inquiry, chaired by Lord Phillips, concluded that the scrapie theory as the origin of BSE was mistaken, and announced the view that BSE occurred naturally, i.e. by spontaneous mutation.

In response to this report by the Philips Inquiry, a review committee on the origins of BSE (chaired by Professor Gabriel Horn and mainly consisting of experts in transmissible spongiform encephalopathy) announced the opinion that the scrapie origin theory could still not be ruled out. Their report also made detailed discussions on the other theories proposed for the origin of BSE, i.e. the spontaneous mutation theory, the acinetobacter theory, and the organophosphates theory, and concluded that neither of these was substantiated by scientific evidence. It also concluded that the theory of an origin in African wildlife was impossible to verify.

In parallel with the investigation into the cause of the infection, meanwhile, researchers at Oxford University made an epidemiological study on forecasting outbreaks of BSE and its human variant, Creutzfeldt-Jakob Disease (CJD).

3.2 Netherlands

The Dutch General Inspection Service (Algemene Inspectiedienst: AID) posited 5 hypotheses for 30 cases of BSE infected cattle, and verified the source and routes of BSE infection in the Netherlands. Its results were as follows.

Infection of BSE in Dutch cattle arose because MBM contaminated with the BSE agent became cross-contaminated in compound feed factories, and was mixed with compound feed that was fed to cattle. The BSE agent was most probably present in MBM produced in the UK, where it had not been sufficiently heat treated.

This correlation with cross-contamination is supported by the fact that, in the BSE-affected area in the Netherlands (the "BSE Triangle"), a large market share was taken up by feed factories that did not rigorously separate the manufacture of ruminant feed from that of other livestock feed.

3.3 Denmark

The Danish Veterinary and Food Administration (VFA) has conducted a detailed survey on feed supplied to cattle, leading to the conclusion that MBM cannot be ruled out as a cause. In one case of BSE infection, the possibility was also confirmed that pig feed using MBM as an ingredient may have been fed to cattle. The VFA posits the following 5 possible scenarios whereby feed may have become contaminated with banned animal proteins.

(1) Inadequate cleaning of trucks and storage tanks
(2) Inadequate cleaning of oil tanks and feed mixing sections
(3) Use of contaminated MBM in feed manufacturing processes
(4) Admixture of pet food with contaminated MBM
(5) Admixture of old feed using MBM in farms and feed factories

Live cattle had been imported from the UK between 1980 and 1989, and the possibility that these were processed into MBM cannot be ruled out.

On investigating milk replacer for calves as a possible cause of BSE infection, moreover, it was confirmed that milk replacer imported from Germany, a BSE-affected country, was used in 10 out of 13 cases of BSE infection. This was considered as one possible cause.

Chapter 2 Epidemiological Analysis

1 Purpose

Epidemiology is a science that investigates the cause of occurrence of diseases. When making an epidemiological study, possibilities are raised as hypothetical causes, based on descriptive epidemiology (gathering and arrangement of information on the outbreak of a disease). The cause of the disease is then postulated by examining the probability of these hypotheses, while factors that should be taken into account in future preventive measures are identified.

This BSE Epidemiological Study Group attempted to identify the sources and routes of infection for the 7 cases of BSE-infected cattle confirmed in Japan so far, using epidemiological approaches based on investigation data obtained to date. Another purpose of the study was to make the investigation results useful in forecasting future BSE outbreaks and managing risk.

Since only 7 cases of BSE have been discovered so far, we adopted the methods of hypotheses and verification, and case-control study. For the former, we listed hypotheses of possible infection sources and routes, and verified the probability of each. For the latter, we made a statistical analysis of infection factors on affected and non-affected farms.

In addition to this, we also conducted quantitative risk assessment based on assumed infection route models. The purpose of this was to serve not only in verifying past outbreaks but also as a basis for risk management and estimating the future risk of outbreaks.

2 Hypotheses on infection sources and infection routes, and their probability

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The prevailing theory on the BSE agent is the "prion theory". This identifies the BSE agent as an abnormal prion protein that is derived from normal prion protein by conformational changes. However, much is still unknown about the agent, its mechanism of manifestation, and other details, and a method of antemortem diagnosis has yet to be established. This makes it difficult to gain an accurate estimation of the level of spread of this disease. The long average incubation period of 5 years, moreover, greatly hinders the identification of infection sources. MBM derived from infected cattle played a major part in spreading the disease in the UK. This much is evident from the fact that numbers of confirmed cases decreased dramatically after a ban on feeding MBM to cattle. France, Ireland, and other countries with a high incidence of BSE, meanwhile, tend to import large amounts of MBM and live cattle from the UK, and these are considered highly likely to have been the sources of infection.

In the findings made so far, the import of contaminated MBM and infected cattle is thought to pose the highest risk as the cause of BSE outbreaks in hitherto unaffected areas. In the EU's Geographical Risk of Bovine Spongiform Encephalopathy (GBR), the import of MBM and live cattle from BSE-affected countries is assessed as a BSE introduction risk.

In the following, we will analyze and discuss the infection sources and routes of the 7 cases of BSE identified in Japan so far, based on the results of investigations conducted by MAFF.

2.1 Study on the possibility that the source of BSE infection was introduced from overseas

One theory on the origin of BSE is that the agent exists naturally in cattle at a low rate of probability, similar to the sporadic CJD in humans (one case in 1 million). Another is that it originated from scrapie in sheep. So far, however, no evidence to support either of these theories has been found. The Western blot patterns of brain emulsion from the 7 BSE-infected cattle in Japan are of Type 4, the same as those in the UK and other BSE-affected countries. This suggests the possibility that the agent originated in the UK. Therefore, we shall now discuss the possibility that imported cattle, MBM and even animal fat from BSE-affected countries, thought to pose the highest risk, were the source of infection in the 7 Japanese cases, on the assumption that the source of BSE infection was introduced from overseas.

2.1.1 Live cattle imported from BSE-affected countries

Cattle imported from BSE-affected countries are suspected of being the infection source for the 7 cases identified in Japan so far, in view of chronological considerations. These cattle are thought to have been imported from the UK, Germany and Canada.

2.1.1.1 Cattle imported from the UK

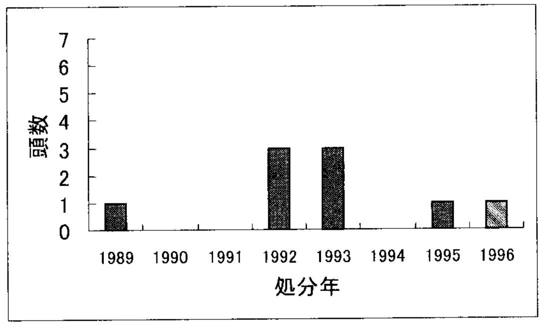
2.1.1.1.1 14 dairy cattle imported to the Kanto region

5 dairy cattle born in the UK in 1979-1980 were imported to the Kanto region in 1982. They were culled between the years 1984 and 1989. As to the reasons for culling, only one (a cow that suffered from postnatal astasia and was culled in 1987) was suspected of having a nervous disorder. Besides this, 3 of the 5 cattle are known to have been processed at rendering plants in the Kanto region.

In the UK, 143 cattle born in 1979-1980 have so far been identified as BSE-infected. The undetected number of infected cattle is thought to be even higher. Even so, the spread of the infection is estimated to have been smaller at that time than at the peak of the outbreak, when 37,000 cases were identified among cattle born in 1987.

Apart from these 5, another 9 dairy cattle born in the UK in 1985-1986 were imported to the Kanto region in 1987. Of these, 8 were processed at rendering plants in Kanto. The other was tested for BSE, proving negative, before being incinerated. The cattle were mainly culled in 1992 and 1993, though some were culled in 1995. The reasons given for culling these imported cattle offer no suspicion of a disease displaying nervous symptoms.

In the UK, 30,000 cattle born in 1985-1986 were identified as being infected with BSE. Although this figure is less than that of the peak among cattle born in 1987, it is speculated that, if we include those not clinically identified, a significant number of cattle were also infected.

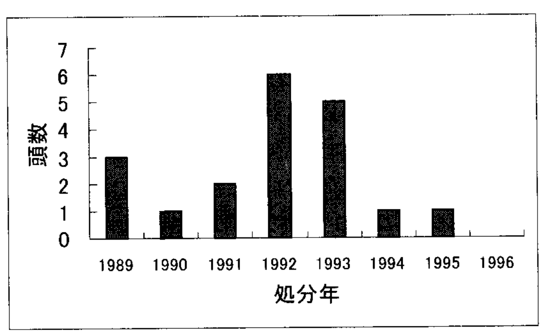


1.Number of cattle 2.Year culled
Number of imported UK cattle culled by year
(Cattle imported in 1987; MAFF survey)

The cow that was culled in 1996 was incinerated after proving negative in BSE testing, and is not considered a source of infection for the 7 identified cases. The ban on the use of MBM in the UK started in 1988, and it is highly likely that cattle born in 1985-1986 were fed MBM in the UK. In fact, many cases of BSE have been identified among cattle born in this period. Moreover, only those that manifested clinical symptoms were identified as infected in the UK at the time, and it is speculated that many more cattle were latently infected.
Many of these cattle imported from the UK were rendered in the Kanto region after being culled. The possibility cannot be ruled out that the MBM thus produced was fed to cattle as an ingredient in compound feed, through cross-contamination, or by other means.
Both cases of BSE infection confirmed in Kanto were born in the spring of 1996. On the hypothesis that the imported cattle culled in October 1995 were infected, and that MBM derived from them was the source of the infection, this would coincide with the Kanto cases in temporal terms. Taking the hypothesis that the imported cattle culled in 1989-1993 were infected, meanwhile, it is possible that MBM derived from them caused the infection in the Kanto cases through recycling.

2.1.1.1.2 19 dairy cattle imported to Kyushu

In 1988, 19 dairy cattle born in the UK in 1985-1986 were imported to Kyushu. These were culled between the years 1989 and 1995, and were rendered in Kyushu.



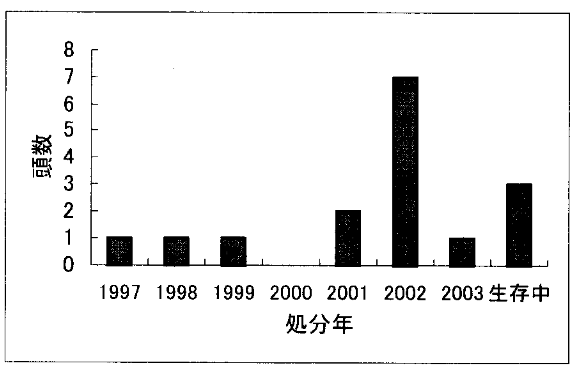
1.Number of cattle 2.Year culled
Number of imported UK cattle culled by year
(Cattle imported in 1988; MAFF survey)

It is possible that the cattle born in the UK in 1985-1986 were infected, as mentioned earlier. As such, the possibility arises that the infection was caused by

MBM derived from these cattle. To date, however, there have been no confirmed cases in Kyushu. There, pigs and chickens are reared in large numbers, and large volumes of MBM are consumed within the region. This makes it unlikely that MBM produced in Kyushu was shipped to far-off regions like Kanto and Hokkaido. Consequently, these cattle are unlikely to have caused the outbreaks in Kanto and Hokkaido. Moreover, the factories that supplied compound feed to the 7 farms with confirmed cases purchased their MBM from rendering plants within their own regions, and not from Kyushu.
Taking the hypothesis that these imported cattle were infected, the possibility exists that they became a source of infection via rendering. But then it would be hard to explain why this happened in Hokkaido and Kanto, but not in Kyushu. Therefore, the cattle imported to Kyushu are thought unlikely to have caused the cases confirmed so far.

2.1.1.2 Cattle imported from Germany

In 1993, 16 dairy cattle born between 1991 and 1993 were imported to Hokkaido from Germany. In Germany, BSE was confirmed in 6 imported cattle between 1992 and 1997, but no case had been confirmed in domestically-bred cattle. In 2000, however, the disease was confirmed in 7 domestic cattle, and surveillance was intensified. More than 100 cases of BSE were confirmed in each of the next two years (125 in 2001 and 106 in 2002). The level of spread of BSE when the cattle were imported is unknown. Nevertheless, the fact that, in Germany, BSE was observed in cattle born in 1992 means that the possibility that these cattle were infected cannot be ruled out – albeit far less likely than the cattle imported from the UK.
Of the 16 imported cattle, 3 are still alive and suggest no suspicion of BSE. Another 8 were subjected to BSE testing but proved negative. Of the remaining 5, 3 have died, but, as the cause of death, there are no findings that suggest suspicion of a disease displaying central nervous signs.



1.Number of cattle 2.Year culled Still alive

Number of imported German cattle culled by year

Bearing in mind that all 7 cases of BSE-infected cattle in Japan were born in or before April 1996, the cow culled in January 1997 could conceivably have been the source of infection, in temporal terms. However, the compound feed factory that supplied all 5 cases in Hokkaido did not handle MBM from the rendering plant where this cow was processed. Meanwhile, taking the hypothesis that cattle infected by MBM derived from this cow became a new source of infection, examples of infection would have occurred from 2000 onwards, making this hypothesis difficult to support in temporal terms. Therefore, the cattle imported from Germany are thought unlikely to have been a source of infection for the 5 cases in Hokkaido.

2.1.1.3 Cattle imported from Canada

Japan imports large numbers of dairy cattle from Canada every year. Canada banned imports of MBM from the UK in 1990, but cattle had been imported from the UK until that time. BSE was confirmed in cattle imported from the UK in 1993. There was no BSE case thereafter until May 2003, when 1 case of infection was confirmed.

In the period from 1986 to 2003, Japan imported 5,210 dairy and other cattle from Canada. The import destinations ranged all over the country, from Hokkaido in the north to Kyushu and Okinawa in the south. The largest proportion went to Hokkaido with 42%, followed by Chugoku and Shikoku with 19% and Tohoku with 13%.

The level of infiltration of BSE in Canada is unknown. If we take the hypothesis that cattle imported from Canada were infected with BSE, the disease could break out in all import destinations. However, it is thought unlikely that the cattle exported to Japan were infected, bearing in mind that only one case has been confirmed among Canadian-bred cattle to date, and that there has been no confirmed case in the USA, which imports around 1.7 million cattle from Canada every year (figure for 2002).

If the cattle imported from Canada were infected, it could be explained that they caused the infection in Hokkaido and Kanto. However, because the level of spread in Canada is unknown, it is difficult at present to compare this with other possibilities.

2.1.2 MBM imported from BSE-affected countries

Since the cases confirmed so far were all born in or before April 1996, we examined the possibility that MBM imported up to 1997 was the source of the infection. Between 1990 and 1997, Japan imported around 200,000 tons of MBM every year, including that used for fertilizer and other non-feed purposes. Of this, exporting countries with confirmed incidence of BSE were Italy, Germany, Austria and Canada.

2.1.2.1 Imported MBM from Italy

656 tons of MBM were imported from Italy in 1987-1990, 20 tons in 1993, and 186 tons in 1995-1997. According to a MAFF survey, it is highly likely that MBM produced before June 1986 was not sufficiently heat-treated. Meanwhile, it has been confirmed that the 20 tons imported in 1993 were derived from poultry, and these were therefore eliminated from the analysis.

In Italy, BSE was confirmed in 2 imported cattle in 1994. There were no further cases until 2001, when the BSE testing system was intensified. Since then, 48 BSE-infected cattle were identified in 2001 and 38 in 2002.

It has been confirmed that most MBM imported between 1995 and 1997 was used for fish feed and pet food in Kyushu. However, the possibility has been pointed out that some of it was used in compound feed. If this MBM was used in compound feed for cattle, or if that used for other animal feed caused cross-contamination with compound feed for cattle, the possibility arises that this imported MBM was the source of infection. But this MBM was shipped to the Chugoku and Shikoku regions, and since it is unlikely that MBM unloaded there would be transported to Hokkaido or Kanto, it would be difficult to postulate this as a direct source of infection in Hokkaido and Kanto.

Nevertheless, while the destinations and other details of MBM imported between 1987 and 1990 are unknown, if MBM from the UK or another BSE-affected country were mixed with this, or if BSE had already spread in Italy, the possibility could not be ruled out that this was a source of infection. The infection source in this case would arise from recycling inside Japan.

2.1.2.2 Other imported MBM

As for BSE-affected countries other than Italy, Japan has imported MBM from Germany, Austria and Canada. Of these, it has been confirmed that the imports from Germany and Austria were either used for purposes other than feed, or were due to statistical errors. Imports from Canada are relatively large in volume, amounting to around 5,000 tons between 1990 and 1997. In Canada, BSE has been confirmed in one beef cow estimated to have been born in 1997. The possibility cannot be ruled out, therefore, that the source of infection was present in the imported MBM. However, the level of spread in Canada is unknown, making it difficult to judge the degree of this possibility at the present stage.

2.1.2.3 MBM import destinations

By import destination in 1995, import volumes were high in Kanto with 51,000 tons (23%), Chubu with 50,000 tons (23%), and Kyushu with 41,000 tons (19%). No imports were landed in Hokkaido. The possibility cannot be ruled out that MBM landed in one region was transported to other regions. However, considering the cost of transportation, there would be no merit in transporting from the import location to a different location. Consequently, most of the MBM is thought to have been consumed in neighboring prefectures.

In a survey of purchasers of MBM used as an ingredient in pig and chicken feed in factories producing compound feed for cattle nationwide (2001), many factories in regions other than Hokkaido used imported MBM as an ingredient. Conversely, no compound feed factory in Hokkaido used imported MBM as an ingredient. From the above, there is thought to be little likelihood that MBM directly imported into Hokkaido became a source of infection.

In the Kanto region, on the other hand, MBM import volumes are large and compound feed factories that handle this are also numerous. Therefore, if contaminated MBM were imported, it is thought possible that it was fed to cattle through direct use of MBM in pig and chicken compound feed, cross-contamination with compound feed for cattle, and so on.

In Kyushu, meanwhile, although the volume of imported MBM is large, the numbers of pigs and chickens reared are also large. Therefore, the proportion used for dairy cattle is small, as is also the proportion of MBM derived from dairy cattle as against the volume of MBM manufactured in Kyushu overall. As such, the possibility of infection spreading through MBM is thought to be lower than in other regions.

2.1.3 Animal fat imported from BSE-affected countries

About 400,000 tons of animal fat are used every year in compound feed as a source of energy supplementation. According to figures for fiscal 2001, the total volume used was around 388,000 tons. This breaks down into about 306,000 tons (78.9%) for chicken feed, about 75,000 tons (19.2%) for pig feed, and about 6,700 tons (1.7%) for cattle feed. Nearly all of this (99.9% in an interview survey on figures for fiscal 2000) is domestically produced, and the ratio of use of imported products is thought to be small. However, the exact figures are unknown. Figures for the import of animal fat used in feed from BSE-affected countries over the last 15 years (the Ministry of Finance's "Japanese Trade Monthly Bulletin") show that 22 tons of "tallow (for use in feed)" was imported from Switzerland in 1989. However, imports of powdered fats (fats coated with casein, etc., and pulverized) cannot be ascertained statistically.
Animal fat itself is not thought to be infectious. If it contains infectious proteins as impurities, however, the possibility that it could become a source of infection cannot be ruled out (though this has yet to be experimentally proved). Nevertheless, much is unknown about the actual import and distribution of animal fat, and it is difficult to analyze from the perspective of imports from abroad. Therefore, we based our analysis and evaluation on surveys of animal fat actually used in the affected farms (to be discussed later).

2.2 Discussion on infection sources by hypothesis and verification

So far, 7 cases of BSE infection have been confirmed in Japan, and detailed surveys have been made of the feed, veterinary pharmaceuticals and others used in the respective breeding farms and affected farms. In 3 of these cases, BSE occurred on farms to which cattle had been moved from their original breeding farms. In all 3 cases, the age on transfer was 2 years or older, and in view of the incubation period of 5 years, it is highly likely that the cattle were infected while still in the breeding farms. Moreover, no reports have been obtained to the effect that the cattle were exposed to MBM or other suspected sources of infection at the affected farms. Considering these points, we based our study on the results of surveys at the breeding farms related to the confirmed cases.
Here, of elements to which the infected cattle could have been exposed, we will raise exhaustive hypotheses for those that could have been the source of BSE infection. We will state the grounds for both supporting and refuting each hypothesis, and evaluate the possibility that they could have been the source of infection.

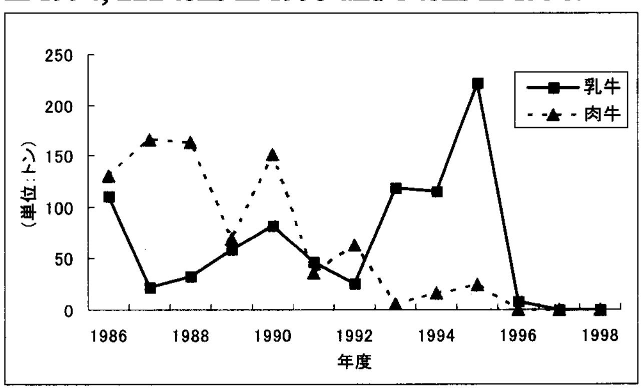
2.2.1 Hypothesis 1: Infection was caused by MBM as an ingredient in compound feed

<u>Factual background</u>

In the UK, reports suggest that compound feed for cattle used to contain 2-5% of MBM, and compound feed for calves a maximum of around 5%. This MBM in compound feed is thought to have been a major cause of the spread of BSE. In other European countries, too, incidence of BSE tends to be high in countries that habitually used MBM in cattle feed. There are reports, for example, that compound feed for cattle contained MBM at a ratio of 1.5% in France and 2.6% in Switzerland.
In the UK, the feeding of ruminant-derived MBM to cattle was banned in 1988, and the number of incidences decreased dramatically as a result. Numerous cases have been confirmed in cattle born immediately after that, however. These are known as BAB (born after ban) cases. They are speculated to have been infected

because stocks of already distributed compound feed containing MBM still remained, or because MBM used in compound feed for pigs and chickens became mixed with compound feed for cattle (cross-contamination).
In Japan, the MAFF recommended restraint in the feeding of ruminant-derived MBM to ruminants in April 1996. Before that, MBM had been used in compound feed for cattle, albeit in small volumes.

- According to investigations so far, there is no evidence that the compound feed given to the 7 confirmed cases contained MBM (the 7 affected farms used a total of 27 types of compound feed, none of which contained MBM).
- Even before 1996, the volume of MBM used in compound feed for cattle was small. The proportion of MBM to all ingredients in compound feed for cattle was a tiny 0.01% for both dairy and beef cattle (according to the Feed Monthly Bulletin, the figures for chickens and pigs in FY1995 and FY1996 were 3% and about 1.5%, respectively).
- In terms of the volumes of MBM used in compound feed manufactured before 1996, MBM was used mainly as an ingredient in chicken and pig feed. The amount used in compound feed for dairy and beef cattle was a mere 0.05% of all MBM used in compound feed. The volume used for dairy cattle was 118 tons in 1993, 115 tons in 1994, 222 tons in 1995 and 8 tons in 1996.



1.(unit: tons) 2.Dairy cattle,Beef cattle 3.FY

Trends in use of MBM in compound feed for cattle

(Commercial Feed Division, Livestock Industry Department, Agricultural Production Bureau, MAFF: Factual Survey on Commercial Feed Prices, etc.)

- Although MBM is said to have been used as an ingredient in compound feed for calves (milk replacer, calf starter, etc.), no detailed records remain concerning the circumstances of its use at that time.
- In an investigation of Japanese rendering plants in 2001, of MBM produced in Japan (including that derived from cattle, swine and poultry), 6.4% was produced by heat treatment satisfying international standards (133°C / 20 minutes / 3 bar), thought necessary to inactivate the BSE agent.

MBM production volumes by treatment conditions in rendering plants

(unit: tons)

Rendering plant	Treatment method	Treatment conditions	MBM production volume	Ratio
Cattle only	Continuous	133/20/3	0	0.0%
		Others	180	0.1%
	Batch	133/20/3	0	0.0%
		Others	13548	5.5%
Combined	Continuous	133/20/3	0	0.0%
		Others	118176	47.9%
	Batch	133/20/3	15888	6.4%
		Others	99012	40.1%

(2001 figures – MAFF survey)

- Viewing movements of compound feed (for all livestock) in FY1996, nearly all feed produced in Hokkaido was consumed inside Hokkaido, and only 165 tons out of 3 million tons produced (0.06%) were shipped to Kanto. Similarly, 90% of feed produced in Kanto was consumed in Kanto, and only 4,800 tons out of 5 million tons produced (0.1%) were shipped to Hokkaido.
- According to an investigation of purchasers of MBM used in cattle compound feed factories throughout Japan (2001), nearly all compound feed factories used MBM from rendering plants within the same region or neighboring regions. No factory was observed to purchase MBM from more remote regions.

<u>Positive findings</u>

- Until the restriction on the use of MBM in April 1996, compound feed containing MBM is thought to have been shipped domestically. Moreover, considering inventories and the period of distribution, it is possible that such feed was given to domestic cattle for a short time after April 1996.
- Of the 7 confirmed cases, 6 were born just before the restriction on use of MBM in April 1996. So far, no case has been confirmed in cattle born in or after May 1996. However, infection has been confirmed in cattle born before that (December 1995). The state of occurrence so far can be explained if we assume that the infection risk decreased as a result of the measures taken in April 1996.
- In the UK, infection is estimated to have been common in calves, and there is thought to be a high likelihood of infection if MBM was used in compound feed for calves.

<u>Negative findings</u>

- MBM is not marked among the ingredients of compound feed given directly to the 7 confirmed cases. Although compound feed companies retain no manufacturing records from that time, it is speculated that these compound feeds did not include MBM, since the Feed Safety Law obliges producers to mark all ingredients used.
- There was no compound feed common to all 7 cases, and there was little movement of compound feed, or MBM used in compound feed, between Hokkaido and Kanto.

<u>Discussion</u>

MBM was not marked among the ingredients of compound feed confirmed to have been given to the 7 cases. If these markings are reliable, a direct relationship with the confirmed cases would be disproved, making this hypothesis difficult to

support. It would also be difficult to explain how the infection occurred from the same source in cattle born at the same time in Hokkaido and Kanto.
Considering the overall volume of MBM consumed for feed, the proportion of MBM used in cattle feed is extremely small. Nevertheless, more than 200 tons were used for dairy cattle in 1995, and a considerable volume has been fed to dairy cattle in real terms. On the hypothesis that contaminated MBM was brought into the country, or was already being recycled inside Japan, infection via this route would be conceivable.
From the above, the possibility exists that BSE infection occurred due to MBM present in compound feed for cattle manufactured before April 1996. However, if we assume that MBM was not used in the compound feed given to the confirmed cases, it is thought unlikely to have been a direct cause of infection.

2.2.2 Hypothesis 2: Infection was caused by MBM present in compound feed due to cross-contamination

Factual background

In the UK, as the main reason why outbreaks of BSE continued in cattle born after the ban on feeding of ruminant-derived MBM to cattle in 1988 (BAB cases), the possibility has been pointed out that MBM used as an ingredient in pig and chicken feed became mixed with cattle feed. This is thought to have occurred during the manufacturing process inside compound feed factories or during the transportation of ingredients.
In Japan, since the recommendation for restraint on feeding ruminant-derived MBM to ruminants in April 1996, guidelines designed to prevent admixture of ruminant-derived MBM to cattle feed in compound feed factories were formulated in June 2001. In October 2001, moreover, following the identification of BSE case in Japan, the import as well as domestic manufacture and shipments of MBM were banned.

- Although there is no compound feed factory common to all 7 cases, at least one of the factories that produced the compound feed given to these cases handled MBM when manufacturing feed for other livestock.

Affected farms that used compound feed obtained from factories handling MBM

Compound feed factory	Affected farms						
	①	②	③	④	⑤	⑥	⑦
Factory A	○						○
Factory B	○	○		○		○	○

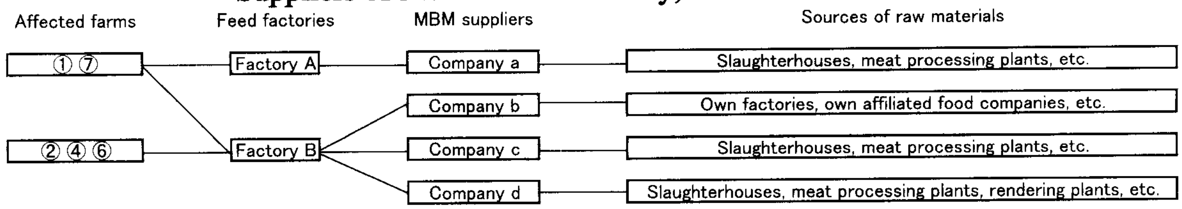
(only factories linked to more than one farm)
(MAFF survey)

- Factory B supplied compound feed to the 5 farms in Hokkaido. An on-site inspection of the factory revealed the possibility of MBM cross-contamination. Meanwhile, 4 of the 5 farms in Hokkaido used compound feed for calves (calf starter) produced by the same factory. The other farm did not purchase compound feed for calves (calf starter).
- Although one factory supplied compound feed to the 2 Kanto cases in common, only one of the farms purchased compound feed for calves. The time of this purchase was after 1999, and the affected animal was aged 3 years or older.

Therefore, it was thought unlikely that it was fed to the affected animal, or that this was the cause of infection.

- The sources of MBM for these factories were domestic slaughterhouses, food companies, and others.

Suppliers of MBM to each factory, and their sources



(MAFF survey)

- We surveyed the feed used in 1996 by 27 farms near the confirmed cases in Hokkaido and 7 farms near those in Kanto, and compared whether or not they had used feed manufactured in compound feed factories A, B and C (which were common to the confirmed cases). As a result, we found there was no difference between the affected farms and the surrounding farms ($p>0.05$) in their ratio of use of compound feed produced in these factories. As an example, the Table below shows ratios of the use of compound feed manufactured in factory B.

Comparison between affected farms and surrounding farms in the ratio of use of compound feed manufactured in factory B

Whole country	Compound feed	
	Used	Not used
Affected farms	5	2
Surrounding farms	16	19

p=0.205

In Hokkaido	Compound feed	
	Used	Not used
Affected farms	5	0
Surrounding farms	15	12

p=0.086

(One-sided P value calculated using Fisher's exact probability test)

- Of around 6.8 million tons of compound feed for cattle manufactured in 1995 (before the restriction on MBM), 17% was produced in factories exclusively for cattle.

Production volumes of compound feed for cattle

(unit: tons)

	FY1995	FY1997
Cattle only	1129464 (17%)	911844 (13%)
Combined	5672774 (83%)	6031317 (87%)

<u>Positive findings</u>

- While susceptibility is said to be high in the early stages of life, 5 of the 7 affected farms used compound feed for calves (calf starter) that could have been cross-contaminated (the other 2 farms did not purchase compound feed for calves).
- There is a compound feed factory common to all 5 cases in Hokkaido and another common to the 2 cases in Kanto.
- Although only 1.5% of dairy cattle shipped from Hokkaido are slaughtered in the Kanto region, the possibility cannot be ruled out that cattle introduced to Kanto from Hokkaido for milking were the source of infection, and that this caused the infection in the Kanto region.
- If compound feed factories independently took some kind of measures to prevent admixture following the recommendation for restraint in April 1996, the risk of infection from this route would be expected to decrease. This could explain why no incidence has been confirmed so far in cattle born after May 1996.

Negative findings

- Although there is a compound feed factory common to all 5 cases in Hokkaido, there was no brand common to all 5 cases, and the cattle are not thought to have been infected by the same production lot.
- If contamination in a compound feed factory is the cause, the fact that infection occurred in cattle born at the same time in Hokkaido and Kanto would mean that there were two sources of infection, and the hypothesis of a common infection source becomes untenable.
- In investigations so far, no imported ingredients were used in MBM, besides meat scraps produced in Australia and New Zealand. Thus, the only possible explanation would be that domestic MBM was contaminated.
- Since dairy cattle from Hokkaido are thought to have been introduced all over the country, this is not consistent with the fact that BSE has only been confirmed in the Kanto region.

Discussion

While it is difficult to explain why cattle born in the same period were infected, it is thought possible that they were infected by consuming cross-contaminated feed. Since there is no compound feed common to the cases in Hokkaido and Kanto, it would be unlikely that the same feed caused infection. Nevertheless, this hypothesis cannot be completely ruled out, as cattle from Hokkaido are also rendered in Kanto and more than one batch of feed may have been contaminated. From the above, there are no grounds for clearly refuting this hypothesis under present circumstances, and the possibility cannot be ruled out that the cattle were infected by cross-contaminated compound feed for cattle. In this case, it would be highly likely that the 7 cases were not infected by a common source of infection, but that there were separate infection sources in both Hokkaido and Kanto.

2.2.3 Hypothesis 3: Infection was caused by MBM in supplementary feed, or by direct feeding with MBM

Factual background

There are a number of conceivable scenarios whereby farms would feed MBM directly to cattle, including: (1) direct feeding as MBM, (2) feeding of supplementary feed containing MBM (such as vitamin supplements or vitamin-mineral mixed feed, mixed with compound feed mainly to supplement vitamins, minerals and others), and (3) feeding of compound feed for swine or poultry. Such details tend not to be recorded, and are, moreover, difficult to ascertain as the period in question is more than 5 years ago.

- It has not been confirmed that any of the 7 farms fed MBM directly.
- In a nationwide investigation of MBM feeding in 2001, it became clear that animal-derived proteins had been fed to about 5,000 cattle across the country. However, the majority of these were steamed bone meal and blood meal, thought to pose an extremely low risk. Few farms fed MBM or compound feed for swine or poultry. These were fed to only 1,496 cattle.
- Supplementary feed confirmed to have been used by the affected farms did not contain MBM (altogether, the 7 farms used 49 types of supplementary feed from 37 factories, none of which contained MBM).
- Chickens were reared on 2 of the 7 farms. However, these farms did not purchase compound feed for poultry, and fed their chickens with cattle feed.
- Of the 37 factories that produced the supplementary feed used by the 7 farms, only 2 handled MBM. Of these, 1 manufactured feed using MBM on a separate line, posing little likelihood of cross-contamination. The possibility of cross-contamination has been pointed out for the other factory (factory B), however.
- Although there was no confirmation of this in the affected farms, it is said that supplementary feed containing MBM, as well as MBM and blood meal as single-substance feed, was being circulated in Hokkaido. However, the suppliers of MBM to these feed manufacturing factories were slaughterhouses and others inside Hokkaido.

<u>Positive findings</u>

- 5 cases of BSE have occurred in Hokkaido, where supplementary feed containing MBM is thought to have been circulated.

<u>Negative findings</u>

- The use of supplementary feed containing MBM by the affected farms has not been confirmed.
- This cannot explain why BSE occurred in cattle born at the same time in Hokkaido and Kanto.
- Supplementary feed containing MBM is said to have been fed mainly to adult cattle to promote milk production, etc., and not to calves, considered more susceptible to infection.

<u>Discussion</u>

If contaminated MBM were fed to cattle at the newborn stage, the risk is thought to be high. According to investigations so far, it has yet to be confirmed that MBM or supplementary feed containing MBM was fed in the affected farms. The same is true of surrounding farms. In an investigation of factories that produced supplementary feed used in the affected farms, the possibility of cross-contamination has only been pointed in a very few cases, and the likelihood of cross-contamination of compound feed is speculated to be relatively small. The possibility cannot be ruled out that supplementary feed containing MBM, said to have been circulated in Hokkaido, was the source of infection. So far, however, no positive evidence has been found to support the suggestion that these supplementary feeds played any part in the BSE cases.

From the above, it is thought unlikely that supplementary feed caused the infection in the 7 cases.

2.2.4 Hypothesis 4: Infection was caused by MBM present in pet food

Factual background

MBM is also used in some pet foods as a source of animal protein. If these were fed to cattle by mistake, the possibility of infection could not be ruled out.

- All 7 affected farms reared cats or dogs, but only 3 of the farms (breeding farms) purchased cat food or dog food.
- It has not been confirmed that these farms fed pet food to cattle.

Discussion

If pet food containing MBM were fed to cattle, the cattle could become infected. However, in the absence of deliberate feeding to cattle, it is thought unlikely that the infection could occur in all 7 cases by some sort of accident.

From the above, while infection could theoretically have taken this route, it is thought highly unlikely that the infection was actually caused in this way.

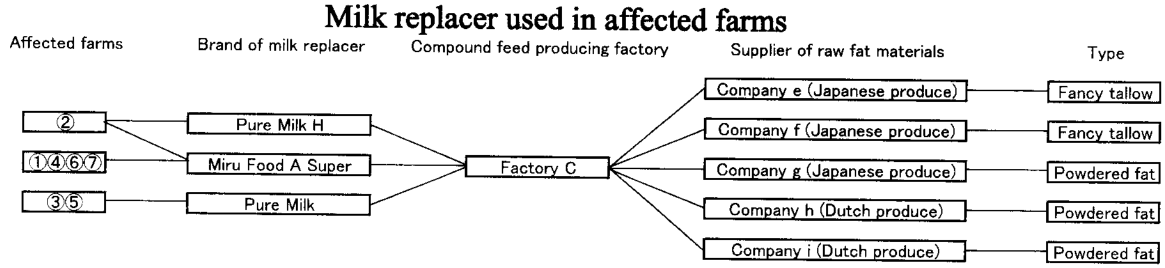
2.2.5 Hypothesis 5: Infection was caused by animal fat (tallow) present in milk replacer

Factual background

Milk replacer contains animal fat (tallow). The origin of this tallow can be divided into that produced via the rendering process, and that derived from fatty tissue obtained from carcasses and food processing. While fats are not thought to be infectious in themselves, if they contain infectious proteins as impurities, they could become a source of infection. In its risk assessment of fatty tissue, the EU's Scientific Steering Committee has published the opinion that fatty tissue is unlikely to pose a risk of infection as long as the intestinal lymph nodes and mesenteric tissue are removed at the time of slaughter. In fats derived from rendering, it has been reported that infectivity has not been detected in any method of rendering in model experiments. Therefore, infectivity via tallow is regarded as being caused by contamination with nerve tissue or intestinal lymph node tissue. In Germany and some other countries, tallow derived from rendering is suspected of being a source of infection.

- All 7 cases were fed with milk replacer produced by factory C, albeit under different brands.

Milk replacer used in affected farms



- The factory used animal fat from the Netherlands (a BSE-affected country) to manufacture milk replacer.
- Dutch-produced animal fat does not originate from rendering plants but from fatty meat or abdominal cavity fatty tissue extracted when dismembering carcasses at slaughterhouses. It is said to be highly refined, with an impurity content of 0.02 or

less. This is well within the standard of 0.15% which the OIE recognizes as "protein-free".

- The factory did not import any other mammalian-derived protein ingredients from BSE-affected countries. Therefore, the possibility of cross-contamination is hard to envisage.
- Domestically-produced animal fat also originated from fatty tissue extracted from carcasses, not from rendering, and was highly refined.
- Milk replacer from this factory was also commonly used in surrounding farms, and no tendency can be discerned whereby only the affected farms used them ($p>0.05$).

Comparison between affected farms and surrounding farms in the ratio of use of milk replacer manufactured in factory D

Whole country

	Compound feed	
	Used	Not used
Affected farms	7	0
Surrounding farms	27	8

p=0.199

In Hokkaido

	Compound feed	
	Used	Not used
Affected farms	5	0
Surrounding farms	23	5

p=0.414

(One-sided P value calculated using Fisher's exact probability test)

<u>Positive findings</u>

- This is the only feed common to all 7 cases.
- It is fed over a short period after birth, and if milk replacer from a specific period were contaminated, the cattle born in that period would be infected.
- It is possible that milk replacer made from Dutch-produced powdered fats shipped on September 26th, 1995, or December 9th, 1996, was fed to all 7 cases.

<u>Negative findings</u>

- The animal fat used was highly refined and did not originate from rendering. Therefore, it is unlikely to have been contaminated with proteins derived from SRM tissue.
- In its risk assessment of fatty tissue, the EU's Scientific Steering Committee suggests that fatty tissue is unlikely to pose a risk of infection as long as the intestinal lymph nodes and mesenteric tissue are removed at the time of slaughter.
- Three different brands of milk replacer were used, and they were not from the same lot.
- No contamination with animal protein was confirmed during on-site inspections of the source factory in the Netherlands.
- In an investigation of infection sources in the Netherlands, it was found that milk replacer was not used in 6 out of 30 BSE cases, while the remaining 24 cases used milk replacer produced by various different factories. As such, no connection could be found between milk replacer and outbreaks of BSE.
- There were 4 confirmed cases in farms that used products with a low admixture (0-3.5%) of Dutch animal fat, but only 1 case on a farm that used products with the highest admixture (7-11.25%).
- There have to date been no reports of experimental infection using animal fat.
- The producing factory shipped products widely, to the Hokkaido, Tohoku, Kanto, Hokuriku, and Tokai regions, and enjoyed a 20% national share of milk replacer

sales. These facts make it hard to explain how milk replacer could have caused BSE infection in Hokkaido and Kanto only.

Discussion

On the hypothesis that the 7 cases of BSE were caused by a single, common infection source, the easiest explanation would be that milk replacer was contaminated with the BSE agent. Of the ingredients in milk replacer, animal fat is the one that could be a source of infection, and since Dutch-produced animal fat was used in all 7 farms with confirmed cases, it must have been contaminated with the BSE agent. However, while it cannot be ignored that this milk replacer was common to all 7 cases, no evidence to support this possibility has been found in the factual background to the animal fat used.

2.2.6 Hypothesis 6: Infection was caused by animal fat used in compound feed other than milk replacer

Animal fat is not only used in milk replacer, but is also added to various compound feeds. On the hypothesis that animal fat was infectious, the possibility cannot be ruled out that cattle were infected by compound feed containing animal fat as an additive.

- Of the compound feed used in the 7 farms with confirmed cases, 6 brands were found to contain animal fat.
- None of these was common to all the confirmed cases, but 3 brands produced by factory B were fed on 4 farms in Hokkaido. The other 3 brands were only used by the farm with the 3rd confirmed case.
- Compound feed containing animal fat was not used on 2 of the affected farms.

Brand of compound feed	Producing factory	Affected farms ①	②	③	④	⑤	⑥	⑦
Miru Food B Flake	Factory B	○						
Miru Food B Green	Factory B		○					○
Miru Food B	Factory B						○	
Kumiai Ex Morlet	Factory D			○				
Hi-Tech 28	Factory D			○				

Use of compound feed containing animal fat

(MAFF investigation)

- The brands common to the 4 farms in Hokkaido were also used in the same way in surrounding farms, and not specifically in the affected farms.

Comparison with surrounding farms

Brand of compound feed	Producing factory	Surrounding farms using the feed ①	②	③	④	⑤	⑥	⑦
Miru Food B, various types	Factory B	2(7)	1(6)	0(2)	4(5)	0(5)	4(5)	4(5)

* Figures in brackets are the numbers of farms investigated

(MAFF investigation)

Discussion

If the animal fat used in compound feed for calves contained proteins derived from SRM tissues as impurities, this could have caused infection. Considering, however, that compound feed containing animal fat was not used by 2 of the farms, the likelihood that these were a source of infection is thought to be even lower than if animal fat was present in milk replacer.

2.2.7 Hypothesis 7: Other causes

2.2.7.1 Infection was caused by MBM in fish meal included in compound feed

Factual background

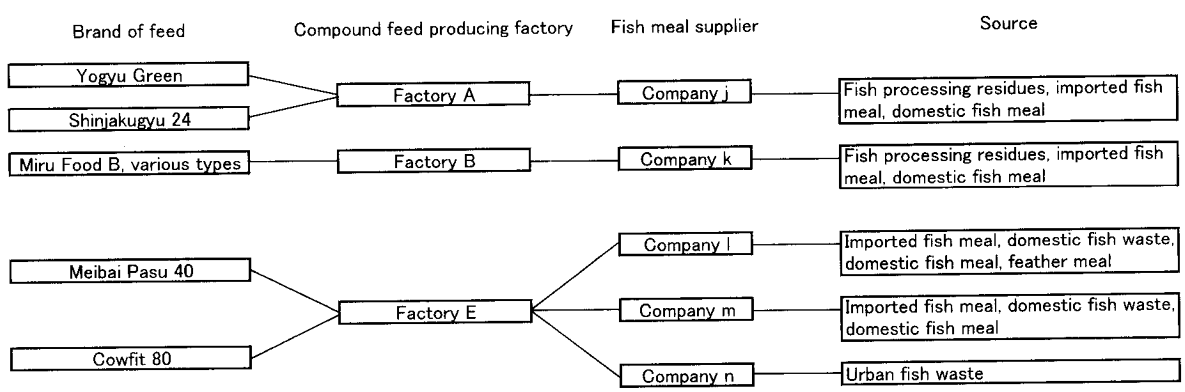
Compound feed containing fish meal was used by 4 of the Hokkaido farms and the 2 Kanto farms. The compound feed used by 2 of the 4 Hokkaido farms was produced at the same factory, but the brands were different. The fish meal itself was manufactured by 5 different companies, only one of which (company m) was confirmed to have handled MBM. Compound feed containing fish feed manufactured by company m was only used in 1 of the Kanto farms, but not in the others.

Mammalian-derived protein has been detected in fish meal at some fish meal producing factories, but this was thought to have been present in food scraps used as an ingredient in the meal.

Brand of compound feed	Producing factory	Affected farms ①	②	③	④	⑤	⑥	⑦
Yogyu Green	Factory A	○						○
Shinjakugyu 24	Factory A	○						○
Miru Food B Flake	Factory B	○						
Miru Food B Green	Factory B		○					○
Miru Food B	Factory B						○	
Meibai Pasu 40	Factory E					○		
Cowfit 80	Factory E					○		

Use of compound feed containing fish meal

(MAFF investigation)



(MAFF investigation)

Discussion

Although compound feed containing fish meal was fed to cattle in 5 of the 7 affected farms, only company m handled MBM in factories where it manufactured fish meal used as an ingredient. Moreover, there was no commonality, as only 1 of the farms with confirmed cases used compound feed containing fish meal from company m. Mammalian protein has been detected in fish meal in a recent investigation, but the possibility has been pointed out that this was present in food scraps used as an ingredient in the meal. In any case, there is nothing in common between the fish meal producing factories used by the affected farms. Moreover, some affected farms did not use compound feed containing fish meal. Therefore, the likelihood of this hypothesis is thought to be extremely small.

2.2.7.2. Veterinary pharmaceuticals

<u>Factual background</u>

Some vaccines, therapeutic drugs, and others are administered directly to cattle. If such veterinary pharmaceuticals contained the BSE agent, infection could be caused by smaller volumes than through oral infection.

In April 1996, Japan banned the manufacture of veterinary pharmaceuticals and others using substances derived from ruminants produced in the UK. In December 2000, the use of ruminant-derived substances (with certain exceptions) produced in other EU countries, Switzerland, and Liechtenstein (and, from June 2001, the Czech Republic) was also banned. Finally, in October 2001, the use of ruminant-derived substances from any country was banned, unless they clearly contain no substances that could be infectious.

Veterinary pharmaceuticals used by more than one confirmed case, and whether they contain ruminant-derived substances

Drugs, vaccines, etc.	Manufacturer	Affected farms ①	②	③	④	⑤	⑥	⑦	Ruminant-derived substances
Koen Selenics	Company o	○	○		○	○	○	○	
Koen E100	Company o	○	○		○				Stearic acid (fat-derived)
Cefamezin QR	Company o		○			○		○	Monostearic acid glycerin, oleic acid methyl, stearic acid (fat-derived)
Rebatio Fluid	Company o		○			○			None
Isotonic Ringer Glucose V for injection	Company o			○		○			None
Pronalgon F injection fluid for animals	Company p	○	○		○			○	None
Panacelan Hi	Company q		○				○		None
Betecillin for Injection	Company r			○		○			Lactose, peptone, skimmed milk (milk-derived)
IBR Vaccine KB	Company s		○				○		Lactose, etc. (milk-derived), serum

(MAFF investigation)

- There were 9 types of veterinary pharmaceuticals that were supplied to more than one of the affected farms. Of these, 4 products contained ruminant-derived substances.
- Of the 7 farms with confirmed BSE cases, 6 used one type of mineral drug produced by company o, and 3 used another type. However, the stearic acid derived from ruminant fat contained in this drug undergoes a considerable degree of treatment in the manufacturing process.
- Antibiotics manufactured by company o, injected into udders during lactation, was used by 3 of the 7 farms. However, these were thought to be used for cattle aged 2 years or more, as a therapeutic drug during lactation. Also, the ruminant-derived fatty acids present in these drugs undergo a considerable degree of treatment in the manufacturing process.
- Antibacterial drugs manufactured by company r were used by 2 of the 7 farms, but the ruminant-derived substances were milk-derived, and their use has not been confirmed in the other 5 farms.

- Vaccines manufactured by company s were used by 2 of the 7 farms, but their use by the other 5 farms has not been confirmed. Considering the sales volume of the vaccines, moreover, if they were the source of the infection, the outbreak would be unlikely to stop at only the 7 cases confirmed so far.

Discussion

If veterinary pharmaceuticals are posited as the source of infection, large numbers of infected cattle should be expected, in view of their sales volumes. This is not consistent with the fact that only 7 cases have been identified so far. Moreover, the only veterinary pharmaceuticals with a high degree of commonality among the 7 confirmed cases are only solid mineral drugs (Koen Selenics, Koen 100) whose effective constituents are ingested orally. The fact that oral ingestion of infection sources has a significantly lower infection rate than direct administration into the body by injection or other means, and the fact that the ruminant-derived constituents present in these products are subjected to a considerable degree of treatment, suggest that these are extremely unlikely to have been the source of infection.

3 Case-control study

(authors: **Yasuharu Yoshida**, Head of the Department of Food Policy & Evaluation, Policy Research Institute, MAFF, and **Mutsuyo Kadohira**, Associate Professor, Nagoya University International Cooperation Center for Agricultural Education.

Information on feed used in the 7 farms with confirmed cases of BSE ("case farms") and 37 farms in surrounding areas ("control farms") was collected by prefectural departments of animal health, via on-site inspections after the outbreak of BSE. We created a database of this information, to which we applied the following statistical analysis. Using the "Case Control Study" approach, we first statistically analyzed the use of feed (risk factors) in two groups, viz. case farms and control farms.

3.1 Data collected

3.1.1 Affected farms ("case farms")

There were 7 farms with confirmed cases. In 3 of these cases (cases 1, 6 and 7), the cattle were bred on a different farm. We surveyed all of these, but used the 7 breeding farms for our analysis (*1).

3.1.2 Surrounding farms ("control farms")

Data on surrounding farms were collected in the areas surrounding the farms with confirmed cases (or the breeding farms, in cases 1, 6 and 7). The number of farms from which data were collected were as follows.

Table 3-1 Number of surrounding farms surveyed

Case	Number of surrounding farms	Location
1	7	Saroma-cho, Hokkaido
2	6	Sarufutsu Village, Hokkaido
3	2	Miyagi Village, Gumma Prefecture
4	5	Onbetsu Village, Hokkaido
5	7	Isegahara City, Kanagawa Prefecture
6	5	Shibecha-cho, Hokkaido
7	5	Yubetsu-cho, Hokkaido
7 farms	37	

However, of the 7 surrounding farms in case 5, data on feed used in 1995 and 1996 (when the BSE outbreak occurred) are unknown for 2 farms (surrounding farms 5-6 and 5-7). These were therefore removed from the analysis.
Thus, for our analysis we used data on the 7 affected farms and 35 surrounding farms, totaling 42 farms in all.

Table 3-2 Number of affected and surrounding farms

	Whole country	Hokkaido	Prefectures
Affected farms ("case farms")	7	5	2
Surrounding farms ("control farms")	35	28	7
Total	42	33	9

3.1.3 Types of data

The main types of data collected from the investigated farms were as follows.

- Number of cattle reared (adults, rearing cattle, calves)

- Types of feed, etc. (milk replacer, calf starter, compound feed, single-substance feed, supplementary feed, roughage, raw milk, additives, silage additives, veterinary pharmaceuticals)
- Feeding stage
- When feeding was started and finished

3.2 Analysis

Independence tests (see Supplementary Discussion: Independence Tests) were conducted (1) by use or non-use, (2) by brand, and (3) by producing factory, for three types of feed, namely (1) milk replacer, (2) calf starter and (3) compound feed.

As some brands of feed were not distributed outside Hokkaido, the tests on these brands were based only on data from Hokkaido (the same applies to producing factories).

3.2.1 Milk replacer

We conducted the following three tests for milk replacer.

(1) By use or non-use of milk replacer
(2) By brand of milk replacer
(3) By milk replacer producing factory

3.2.1.1 Test by use or non-use of milk replacer

The results of this test are shown below. These differ from those shown on page 27, which concern the use of milk replacer produced in the Takasaki Factory of Scientific Feed Laboratory Ltd. and incidences of BSE. These results, on the other hand, include milk replacer produced elsewhere.

Table 3-3 Milk replacer used and incidences of BSE

Whole country	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Milk replacer used	7	32	39
Milk replacer not used	0	3	3
Total	7	35	42

Occurrence probability of contingency table: $0.57012 > 0.05$
One-sided test P value: $0.57012 > 0.05$

The data for Hokkaido alone are as follows.

Table 3-4 Milk replacer used and incidences of BSE in Hokkaido

Hokkaido	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Milk replacer used	5	25	30
Milk replacer not used	0	3	3
Total	5	28	33

Occurrence probability of contingency table: $0.60044 > 0.05$
One-sided test P value: $0.60044 > 0.05$

With the results of tests for the whole country and Hokkaido alone, the null hypothesis (milk replacer and occurrence of BSE are independent) cannot be disproved at a significance level of 5%.

3.2.1.2 Test by brand of milk replacer

The four main brands of milk replacer fed to cattle were as follows: "Pure Milk" (except Hokkaido), "Premium Meiraku", "Miru Food A Super" (Hokkaido only), and "Miru Food A Special" (Hokkaido only). Of these, "Miru Food A Super" and "Miru Food A Special" used the same ingredients and were therefore analyzed as the same product (*2).

The contingency table and test results for "Miru Food A Super" and "Miru Food A Special", used in the greatest number of farms, are as follows. These data are for Hokkaido only.

Table 3-5 Use of Miru Food and incidences of BSE

Hokkaido	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Miru Food A used *1)	5	23	28
Miru Food A not used *2)	2	3	5
Total	7	26	33

*1) Either "Miru Food A Super" or "Miru Food A Special" was used.
*2) Neither "Miru Food A Super" nor "Miru Food A Special" was used.

Occurrence probability of contingency table: 0.23005 > 0.05
One-sided test P value: 0.94815 > 0.05

For other brands, we will give the results only.

- Pure Milk (whole country)

Occurrence probability of contingency table: 0.16157 > 0.05
One-sided test P value: 0.18752 > 0.05

- Pure Milk (prefectures only)

Occurrence probability of contingency table: 0.27778 > 0.05
One-sided test P value: 0.27778 > 0.05

- Premium Meiraku (whole country)

Occurrence probability of contingency table: 0.36280 > 0.05
One-sided test P value: 0.42988 > 0.05

These results show that the null hypothesis (i.e. "Using this brand of milk replacer did not cause incidences of BSE") cannot be disproved at a significance level of 5%.

3.2.1.3 Test by milk replacer producing factory

The milk replacer produced in the Takasaki Factory of Scientific Feed Laboratory Ltd. (referred to below as "Takasaki") accounts for the majority of data by milk replacer producing factory. The factory's main products are "Miru Food A Super" and "Miru Food A Special", though it also produces "Pure Milk" (*3).

The results of data on the use of milk replacer produced in Takasaki are as follows. These data are the same as those shown on page 28.

Table 3-6 Use of milk replacer produced in Takasaki and incidences of BSE

Whole country	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Takasaki milk replacer used	7	27	34
Takasaki milk replacer not used	0	8	8
Total	7	35	42

Occurrence probability of contingency table: 0.19941 > 0.05
One-sided test P value: 0.19941 > 0.05

The null hypothesis, i.e. that use of milk replacer produced by the Takasaki Factory did not cause incidences of BSE, cannot be disproved at a significance level of 5%.

From the above, the results of several tests on the use of milk replacer and BSE incidence all showed that, for both brands and producing factories, the null hypothesis could not be disproved at a significance level of 5%. Therefore, no statistical evidence was obtained to support a causal relationship between milk replacer and BSE incidence.

3.2.2 Calf starter

We conducted the same three tests for calf starter as for milk replacer, namely:

(1) By use or non-use of calf starter
(2) By brand of calf starter
(3) By calf starter producing factory

3.2.2.1 Test by use or non-use of calf starter

The results of the test by use or non-use of calf starter are shown below.

Table 3-7 Calf starter used and incidences of BSE

Whole country	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Calf starter used	5	26	31
Calf starter not used	2	9	11
Total	7	35	42

Occurrence probability of contingency table: 0.34639 > 0.05
One-sided test P value: 0.74407 > 0.05

3.2.2.2 Test by brand of calf starter

The four main brands of calf starter were as follows: "Premium Mei Starter", "Miru Food B" (Hokkaido only), "Miru Food B Green" (Hokkaido only), and "Miru Food B Flake" (Hokkaido only). The test results for "Miru Food B Green", used in the greatest number of farms, are as follows.

Table 3-8 Use of Miru Food B Green and incidences of BSE

Hokkaido	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Miru Food B Green used	2	13	15
Miru Food B Green not used	3	15	18
Total	5	28	33

Occurrence probability of contingency table: 0.36101 > 0.05
One-sided test P value: 0.77050 > 0.05

For other brands, we will give the results only.

- Premium Mei Starter

Occurrence probability of contingency table: 0.28455 > 0.05
One-sided test P value: 0.30894 > 0.05

- Miru Food B

Occurrence probability of contingency table: 0.34641 > 0.05
One-sided test P value: 0.39956 > 0.05

- Miru Food B Flake

Occurrence probability of contingency table: 0.30261 > 0.05
One-sided test P value: 0.91426 > 0.05

3.2.2.3 Test by calf starter producing factory

Products of the Hokuren Kumiai Shiryo Kushiro-Nishi Minato Factory (referred to below as "Kushiro-Nishi Minato") account for the majority of data by calf starter producing factory. The results of the test for this factory are as follows. Since the products of this factory are not thought to be distributed outside Hokkaido, we analyzed the data for Hokkaido only.

Table 3-9 Use of calf starter produced in Kushiro-Nishi Minato and incidences of BSE

Hokkaido	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Kushiro-Nishi Minato synthetic milk used	4	17	21
Kushiro-Nishi Minato synthetic milk not used	1	11	12
Total	5	28	33

Occurrence probability of contingency table: 0.30261 > 0.05
One-sided test P value: 0.38835 > 0.05

For other factories, we will give the results only.

- Kanto Kumiai Chemical Industry – Akagi Factory

Occurrence probability of contingency table: 0.30261 > 0.05
One-sided test P value: 0.38835 > 0.05

From the above, the results of several tests on the use of calf starter and BSE incidence all showed that, for both brands and producing factories, the null hypothesis could not be disproved at a significance level of 5%. Therefore, no statistical evidence was obtained to support a causal relationship between calf starter and BSE incidence.

3.2.3 Compound feed

Since various types of compound feed were used by all the farms, it would be meaningless to test simply by use or non-use. Moreover, many brands of compound feed are used in various situations for different purposes, and unlike milk replacer or calf starter, there are no "main brands". For compound feed, therefore, we only analyzed the producing factories.

Factories whose products were used as compound feed by both affected farms and surrounding farms were as follows.

Hokuren Kumiai Shiryo – Kushiro-Nishi Minato Factory (referred to below as "Kushiro-Nishi Minato") (Hokkaido only) 15 farms
Mercian – Tomakomai Factory ("Tomakomai") (Hokkaido only) 12 farms
Hokuren Kumiai Shiryo – Kitami Factory ("Kitami") (Hokkaido only) 9 farms
National Federation of Dairy Cooperative Associations (Zenrakuren) – Kushiro Feed Factory ("Kushiro") (Hokkaido only) 7 farms
Nihon Nosan Kogyo – Otaru Factory ("Otaru") (Hokkaido only) 7 farms
Kanto Kumiai Chemical Industry – Akagi Factory (prefecture only) 3 farms

The contingency table and test results for the Kushiro-Nishi Minato data are as follows.

Table 3-10 Use of compound feed produced in Kushiro-Nishi Minato and incidences of BSE

Hokkaido	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Kushiro-Nishi Minato compound feed used	3	12	15
Kushiro-Nishi Minato compound feed not used	2	16	18
Total	5	28	33

Occurrence probability of contingency table: 0.29332 > 0.05
One-sided test P value: 0.40950 > 0.05

For other factories, we will give the results only.

- Mercian – Tomakomai Factory (Hokkaido only)

Occurrence probability of contingency table: 0.30261 > 0.05
One-sided test P value: 0.91426 > 0.05

- Hokuren Kumiai Shiryo – Kitami Factory (Hokkaido only)

Occurrence probability of contingency table: 0.30701 > 0.05
One-sided test P value: 0.41796 > 0.05

- National Federation of Dairy Cooperative Associations (Zenrakuren) – Kushiro Feed Factory (Hokkaido only)

Occurrence probability of contingency table: 0.23005 > 0.05
One-sided test P value: 0.28190 > 0.05

- Nihon Nosan Kogyo – Otaru Factory (Hokkaido only)

Occurrence probability of contingency table: 0.44094 > 0.05
One-sided test P value: 0.72284 > 0.05

- Kanto Kumiai Chemical Industry – Akagi Factory (prefecture only)

Occurrence probability of contingency table: 0.50000 > 0.05
One-sided test P value: 0.58333 > 0.05

From the above, the results of several tests on the use of compound feed produced in various factories and BSE incidence all showed that the null hypothesis could not be disproved at a significance level of 5%. Therefore, no statistical evidence was obtained to support a causal relationship between compound feed and BSE incidence.

3.3 Summary and discussion

As stated above, in our case-control study, no factor (cause) showing statistical correlation with BSE incidence at farm level was found. In other words, we were unable to identify any specific feed suspected of causing the 7 cases of BSE infection. This is thought to result from a complex mechanism of occurrence that is not suited to case-control study – for example, the fact that BSE only occurs in a tiny fraction (2-3%) even of cattle that were fed with infected feed, and that a statistically significant difference cannot be proved due to the small number of cases (7). In future, therefore, it may be necessary to re-attempt analysis and evaluation if cases of incidence increase any further.

Meanwhile, though not verified in this paper, we also collected information and formed a database for data on non-affected farms selected at random (with their consent) from Hokkaido, Iwate, Aichi and Nagasaki Prefectures, besides the affected farms and surrounding farms. However, these data were compiled at the time of the simultaneous on-site inspections held in FY2001, and were not used in our analysis as they were incomplete in certain respects (e.g. duration of feeding).

In this kind of analysis, it is essential that we collect accurate data not only on affected farms but also on surrounding farms. The data used for the analysis this time were inadequate in this respect, as indicated in the notes below. As a result, some of the investigation data had to be excluded.

For future reference, we will recapitulate on the important data that particularly need to be collected.

Above all else, data on types and brands of feed, amounts fed, and duration of feeding are essential. Besides these, other data such as those on the rearing environment are also important.

These data will need to be collected accurately in order to investigate the cause by epidemiological investigations such as the present one. To this end, it will be essential that farms be instructed to keep accurate records of the relevant information.

If the system of production history tracing ("traceability") that was recently started on a trial basis is developed, it should be possible to collect such data very easily. If such a system were to be developed, it is hoped that, if a problem such this outbreak of BSE were to occur in future, it would be possible to investigate the cause effectively in a short time.

*1: The codes used for affected farms are given as "Case n–1 or –2" according to the sequence of occurrence (n = 1-7). Here, "–1" refers to the farm on which the infected animal was born, and "–2" the farm on which it was raised. Therefore, all cases except 1, 6 and 7 have only "–1", while 1, 6 and 7 also have "–2". The farms analyzed here were those on which the cattle were born, and should therefore be farms "1–1" to "7–1". In case 6, it was discovered that "–1" was the rearing farm and "–2" the breeding farm. Therefore, the codes of the farms analyzed are "Cases 1–1, 2–1, 3–1, 4–1, 5–1, 6–2, and 7–1".

*2: In our analysis of milk replacer by brand, they were found to include brands that were commercialized after the outbreak of BSE ("Mirukun" and "Shin-Miruku", both manufactured at the Takasaki Factory of Scientific Feed Laboratory Ltd.). These data were of course removed from our analysis.

*3: The feed used by one of the surrounding farms in Case 1 (farm code "Surrounding farm 1–7") was marked as "Hokuren". However, since the brand was "Miru Food A Super", it was included in the data for the Takasaki factory (as this brand is not produced in any other factory).
Meanwhile, the milk replacer used in "Surrounding farm 5-4" was marked as "Hai Baby" (manufactured by Nihon Nosan Kogyo) in 1995-1997 and "Pure Milk" (produced at the Takasaki factory) in 1998. Since the analysis targeted 1996 (the year in which the confirmed BSE cases were born), the data for "Pure Milk" in 1998 were excluded from the analysis.

Supplement: Independence Tests

This is a technique whereby we compare "case" and "control" with respect to data obtained from experiments and surveys, and test whether or not there is any significance difference between them in relation to a given factor. The technique will be explained in brief here.
Tests as to whether there is any correlation between two variables based on cross-tabulation (contingency tables) are usually performed using a technique such as the $x2$ (chi-square) test. This method will be explained with reference to the example of "Whether the use of milk replacer produced by the Takasaki Factory of Scientific Feed Laboratory Ltd. causes BSE" (abbreviated below to "use of milk replacer and BSE incidence").
The contingency table for this problem, obtained from the survey results this time, is as follows (table on the left of page 28; unit – farms).

Whole country	Affected farms ("case farms")	Surrounding farms ("control farms")	Total
Milk replacer used	7	32	39
Milk replacer not used	0	3	3
Total	7	35	42

H0 (null hypothesis): "There is no correlation between the use of milk replacer and BSE incidence" (i.e. the two are independent).

If this null hypothesis can be disproved at a fixed level of significance (normally 5%), we may posit that "the use of milk replacer causes BSE".

(1) We would usually apply the $x2$ (chi-square) test (*1).
The value of $x2$ is 1.97647, while the one-sided probability (P-value) of $x2$ distribution at a single degree of freedom is 0.160. This is larger than 5% (probability 0.05).
In other words, the null hypothesis cannot be disproved at a significance level of 5%.
However, if any cells have an expected frequency (occurrence probability of each cell x sample size) of 5 or less, it would be inappropriate to use the $x2$ test. With these data (and all other data tested this time), there are cells with an expected frequency of 5 or less. Therefore, we conduct tests using Fisher's direct calculation method, generally used when some cells in a 2x2 contingency table have an expected frequency smaller than 5 (*2).

(2) Using Fisher's direct calculation method, the occurrence probability of this contingency table would be 0.199.
The combined probability (P-value) by one-sided tests including this contingency table would also be 0.199, and, again, the null hypothesis cannot be disproved at a significance level of 5%.
As a result of the above, we adopt (cannot disprove) the null hypothesis.

(3) The conclusion is that "the use of milk replacer does not cause BSE", or, to be more exact, "There cannot be said to be any correlation between the use of milk replacer and BSE incidence".

(4) This is a conclusion based on the investigation data collected. In this kind of independence test, the conclusion could change if more data were collected.

(5) It should be stressed that this result is purely based on data from the 42 farms collected this time.

*1: The $x2$ (chi-square) test
The contingency table is written as follows.

		Factor B				
		B1	B2		Bk	Total
Factor A	A1	o11	o12		o1k	o1.
	A2	o21				o2.
				oij		
	Am	om1			omk	om.
	Total	o.1	o.2		o.k	n

Where oi. = Σjoij
o.j = Σ ioij

The expected frequency of each cell is therefore $Eij = \frac{oi.}{n} \bullet \frac{o.j}{n} \bullet n = oi. \bullet o.j/n$

Value of $x2 = \Sigma_i \Sigma_j (oij - Eij)^2 / Eij$

Assuming that the expected frequency is sufficiently large (normally 5 or more) and n is also sufficiently large, the value of $x2$ would be in accordance with the $x2$ distribution at a degree of freedom of (m – 1) x (k – 1). Therefore, the probability (P-value) is calculated from this. If the calculated P-value is below a given level of significance (normally 5%), the null hypothesis that "Factor A is not the cause of Factor B" is disproved, and the opposite hypothesis, i.e. "Factor A is the cause of Factor B", is adopted.

*2: Test using Fisher's direct calculation method
Given the following 2x2 contingency table:

	B1	B2	Total
A1	a	b	a+b
A2	c	d	c+d
Total	a+c	b+d	n

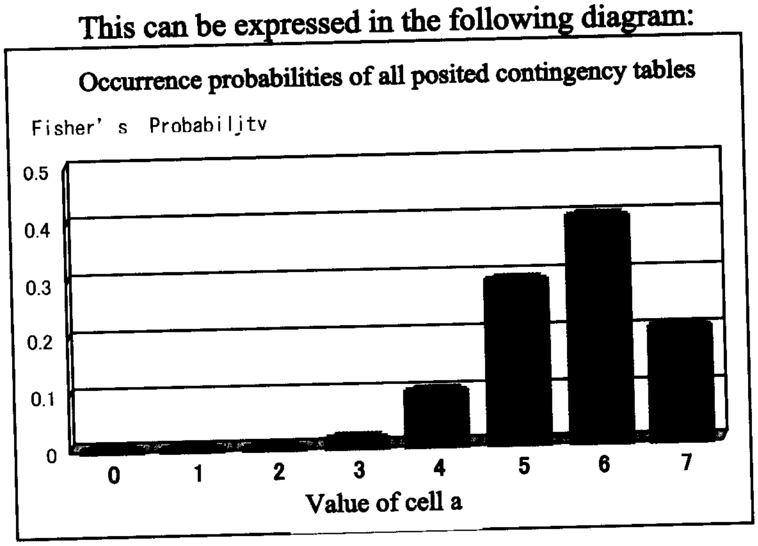
When attributes A and B are independent, the marginal frequency (a+b, c+d, a+c, b+d) is constant. Based on these conditions, the conditional probability P0 (whereby the frequency of each cell is a, b, c, and d) would be

$$P0 = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{n!} \bullet \frac{1}{a!b!c!d!}$$

In other words, the probability that the above contingency table would occur, based on the given marginal frequency, is P0.
In one-sided tests using Fisher's direct calculation method, the occurrence probabilities of all contingency tables obtained by shifting the figures from a given contingency table to the extremes (i.e. making a in the above table large and c small) are calculated and totaled, giving the total P-value. If this P-value is below the level of significance (for example, 5%), the null hypothesis is disproved.
This test process will be explained using the example of the use of milk replacer and BSE incidence.
Since the marginal frequency in a 2x2 contingency table is constant, once the value of cell a (the intersection between Factor A1 and Factor B1 in the table above) is decided, the values of the other 3 cells (b, c, and d) are decided automatically (single degree of freedom).
Since marginal frequency a+c in this contingency table is 7, all contingency tables in which the value of cell a is shifted from 0-7, as well as their occurrence probabilities, can be calculated as follows. Here, a=7 represents the actual data, i.e. the observed value.

	a	b	c	d	Occurrence probability	Cumulative probability	x2 value
	0	34	7	1	0.00000	0.00000	35.70000
	1	33	6	2	0.00004	0.00004	24.21176
	2	32	5	3	0.00116	0.00120	14.94706
	3	31	4	4	0.01553	0.01673	7.90588
	4	30	3	5	0.09626	0.11299	3.08824
	5	29	2	6	0.28879	0.40178	0.49412
	6	28	1	7	0.39881	0.80059	0.12353
(Observed value)	7	27	0	8	0.19941	1.00000	1.97647

This can be expressed in the following diagram:



The test used this time was a one-sided test, to test the hypothesis that "Using milk replacer causes BSE". The value of 7 for cell a is the observed value. Because there is no contingency table with a more extreme value than this, the totaled P-value of the one-sided test concurs with the occurrence probability of the observed value. In other words, the P-value in the one-sided test is 0.19941, and the null hypothesis cannot be disproved.

Although the one-sided test can be used when the directionality of the test is predetermined, with a two-sided test the opposite side also needs to be totaled. In a two-sided test (i.e. a test to determine "whether or not there is a correlation between the use of milk replacer and incidences of BSE") the occurrence probabilities of contingency tables in the opposite extremity are also added. There are a number of ways of doing this, including:

(1) Totaling all the occurrence probabilities of contingency tables in which the occurrence probability is smaller than the observed contingency table

(2) Totaling all the occurrence probabilities of contingency tables whose $x2$ values are larger than the observed contingency table

In the above example, this would mean, in both (1) and (2) above, totaling the occurrence probabilities of contingency tables in which a = 0, 1, 2, 3, and 4, giving a P-value of 0.31240.

When using the one-sided test, all the occurrence probabilities of contingency tables in a more extreme direction would also be added, in addition to the occurrence probability of the observed contingency table. In this case, therefore, the P-value would always be higher than the occurrence probability of the observed contingency table. Consequently, if the occurrence probability calculated from the observed contingency table according to Fisher's method does not exceed 0.05, we know at this stage that the null hypothesis cannot be disproved at a significance level of 5%. All the examples in this case fall into this category.

4 Results and discussion of quantitative analysis using infection route models (risk analysis)

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4.1 Basic principles

4.1.1 Objectives

In September 2001, the first case of BSE infection in Japan was confirmed, and 7 cases have been identified so far. In this analysis, the primary objective is to clarify the likely contamination routes for the 7 cases of BSE-infected cattle born between the end of 1995 and the first half of 1996. Another objective is to study the scale (number of infected animals) and susceptibility to occurrence concerning a number of scenarios for the risk of introduction of the BSE agent into Japan (live cattle, MBM, animal fat) and the risk of exposure to domestically produced cattle in Japan, including these BSE-positive cases. Finally, a further objective is to verify these scenarios based on surveillance data obtained so far, create models that are as realistic as possible, predict future outbreaks of BSE based on these models, and put this to use in epidemiological research and risk management in the event of an outbreak.

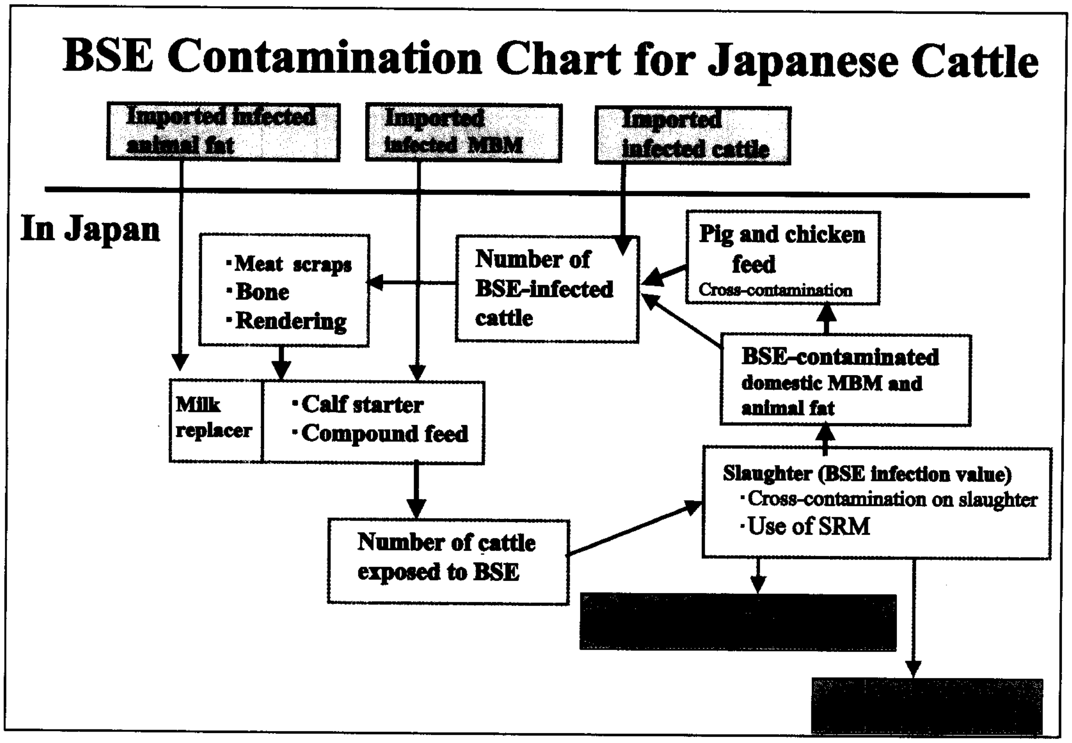
4.1.2 Approaches

We created scenarios for assessing the risk of the BSE agent introduced into Japan, assessing the risk of exposure to domestically produced cattle in Japan, and the possibility of infection spreading among domestic cattle, and evaluated the results. We analyzed the evaluated models as quantitatively as possible. Moreover, since BSE has a long incubation period (5 years on average), the possibility cannot be ruled out that, even though the contamination route was closed off in October 2001, already infected cattle could manifest the disease in future. Therefore, we also predicted the scale of BSE infection that could occur in future.

4.2 Concepts

4.2.1 BSE contamination chart for Japanese cattle

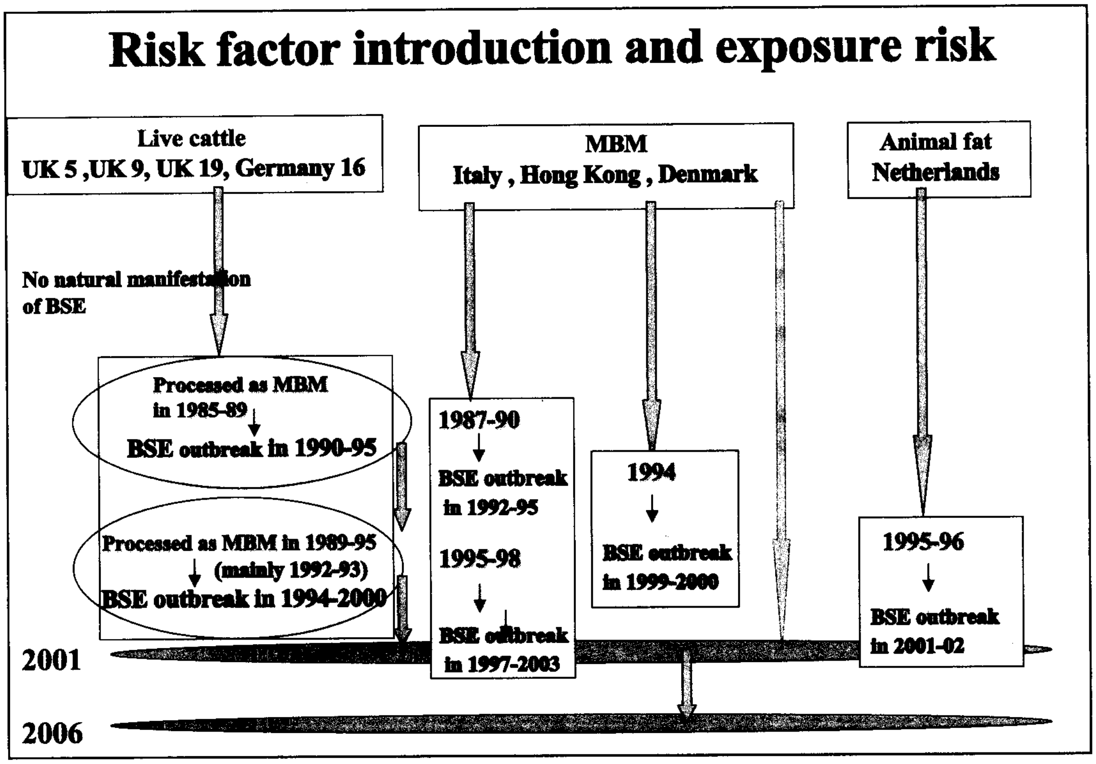
Basically, there are three conceivable routes for introduction of the BSE agent from overseas. These are imported infected cattle, contaminated MBM, and contaminated animal fat. The possibility is also suggested that imported infected cattle in the form of contaminated MBM, or contaminated imported MBM and animal fat, were distributed, variously, through milk replacer, calf starter, compound feed, and so on, and thereby infected domestic cattle before October 2001, when measures to ban the import and manufacture of MBM were imposed. Another factor that should be taken into account is contamination due to the use of specified risk material (SRM) derived from domestically-produced infected cattle in MBM before 2001, when measures to test all slaughtered cattle and to remove SRM in slaughterhouses were first imposed.



BSE contamination chart for Japanese cattle

4.2.2 **Risk Factor Introduction and Exposure Risk**

We created scenarios for the introduction of the above-mentioned risk factors and exposure within Japan, calculated the chronological contamination scale and infection probability for each scenario, and assessed the respective level of risk. If imported live cattle were infected, then used as raw materials for the manufacture of MBM, and this were fed to domestic cattle as feed, it would be expected to take about 5-6 years after the slaughter of the imported cattle before domestic cattle became infected and manifested the disease. Imported MBM and animal fat, on the other hand, are ready-made products. As such, we could estimate about 5 years after import before domestic cattle became infected and manifested the disease. Furthermore, if infected domestic cattle were processed after slaughter and then distributed as MBM, etc., the possibility is suggested that more domestic cattle could be infected 5-6 years after that.



4.2.3 Risk of exposure to BSE agent originating from BSE-positive cattle

The risk of exposure to the BSE agent originating from BSE-positive cattle needs to be assessed comprehensively, taking account of the reduction or inactivation of contamination risk. Assessment should be based on the characteristics of risks due to transmission routes, the oral infection volume in cattle, exposure evaluation, consumption volume and estimated contamination.

4.2.3.1 Risk due to transmission routes

The respective likelihood of oral infection, horizontal infection, vertical infection, and maternal infection have already been studied as transmission routes of the BSE agent. As a result, from epidemiological findings and experiments on the distribution of infectivity in various tissues, horizontal and vertical infection have basically been ruled out. Although no infectivity has been discovered in raw milk, the results of a large-scale experiment on maternal infection in the UK initially suggested a maximum rate of 10%. However, since this includes cross-contamination by MBM, the rate is now thought to be no more than 0.5%. Consequently,oral infection by contaminated feed containing MBM is regarded as the most likely BSE transmission route.

Oral infection by contaminated feed in cattle should be evaluated by the factors of the volume of the BSE agent in contaminated tissue, the infectivity of bovine tissue, and the cumulative effect of multiple ingestion. Experiments have shown that oral infection in newborn calves is caused by 0.1g of the brain of BSE-infected cattle (recent information suggests that the infection is caused even by 0.01g). In cattle with a body weight of 537kg, each animal is estimated to have around 8,000 infectivity units (ID50). The infectivity is mainly found in the brain and spinal cord in natural cases (95%), and in the retina as well. In experimentally infected cases, infectivity has also been found in the peripheral ganglia, distal ileum, and tonsils, in addition to the brain and spinal cord. The cumulative effect due to multiple consumption is regarded as a factor that cannot be ruled out, albeit not occurring with small volumes.

4.2.3.2 Risk due to MBM containing SRM, as well as animal fat, milk replacer, calf starter and compound feed

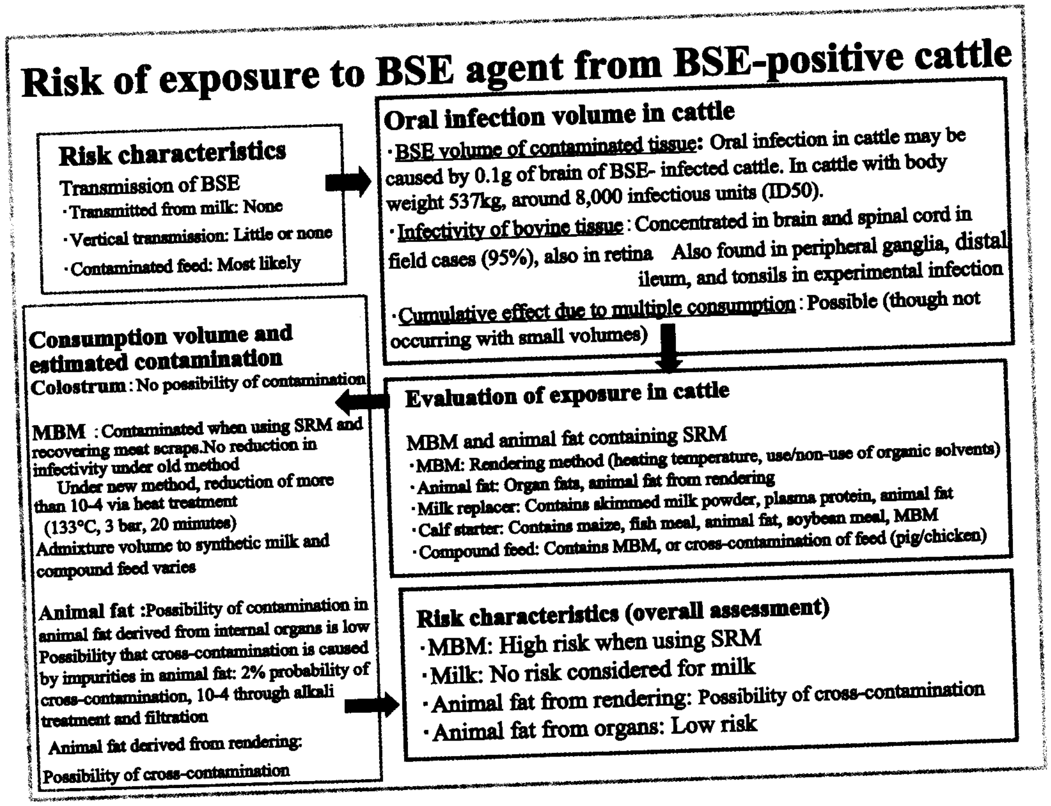
MBM is produced by the method of rendering. In this process, the heating temperature and the use or non-use of organic solvents are important factors that could impact infectivity. Animal fat includes organ fats and fat derived from rendering. Milk replacer contains skimmed milk powder, plasma protein, animal fat, and other ingredients, while calf starter is made from maize, fish meal, animal fat, soybean meal, and others. The inclusion of MBM in compound feed for cattle was stopped following the directive of 1996, but could have been used before that. There were also cases in which blood meal, MBM and others were given as feed supplements, while cattle-derived MBM was used as compound feed for pigs and chicken before the restriction on MBM use in October 2001. It is conceivable that these cross-contaminated compound feed for cattle.

4.2.3.3 Risk due to manufacturing processes

MBM has a very high likelihood of contamination due to the use of SRM. In the manufacture of MBM, hardly any reduction or inactivation of contamination risk could be expected under the old method. With the new method, however, a reduction of more than 10^{-4} in infectivity can be expected by heat treatment (133°C, 3 bar, 20 minutes). Although the possibility of contamination in animal fat derived from internal organs is low, contamination by animal fat derived from rendering cannot be ruled out since this used to contain SRM. In this case, it is possible that cross-contamination was caused by impurities in the animal fat. As for animal fat derived from organs, on the other hand, the infectivity is estimated to decrease by 10^{-4} through alkali treatment and filtration, with a 2% probability of cross-contamination during slaughter and dismembering. The admixture volumes of MBM in compound feed are many and various, and cannot be specified.

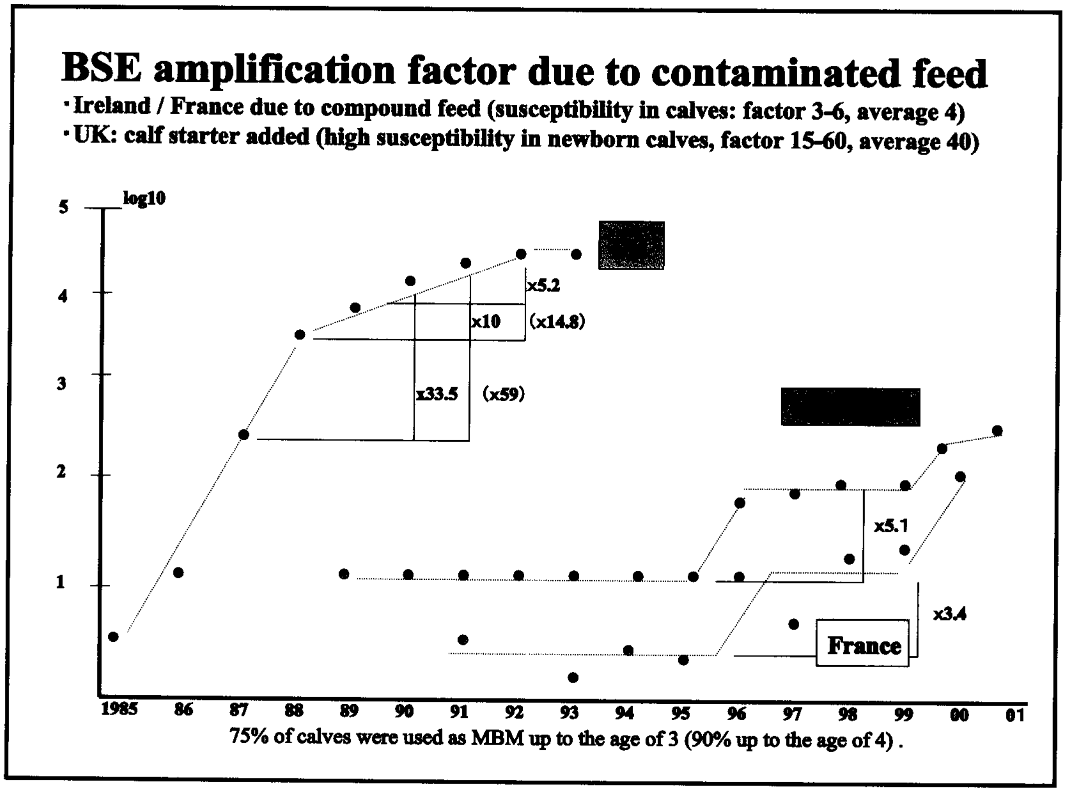
4.2.3.4 Risk characteristics (overall assessment)

MBM presents a high risk when SRM is used, but no risk is considered for milk. Animal fat from organs poses a lower risk than that from rendering, and the possibility of cross-contamination from the latter is conceivable.



4.2.4 BSE amplification factor due to contaminated feed

Contamination within a country is known to spread due to MBM rendering. As stated above, it takes an average of 5 years for BSE to be manifested in cattle that consume MBM manufactured from contaminated animals. Calculating the spread rate of BSE contamination at an interval of 4-5 years, it is thought to be 30-60 times in the UK but only 3-6 times in other EU countries (comparison between pre-1995 and 1996-2000 in Ireland and France). The reason why the rate is 10 times higher in the UK is thought to be that contaminated MBM or others used to be fed to newborn cattle, while in other EU countries contaminated MBM was fed as compound feed to calves. That is, the susceptibility of newborns should be considered as about 10 times that of calves. Since Japan's situation is thought to resemble that of France and Ireland rather than the UK (with a contamination level lower than both of those countries), a multiple of 3-6 times is thought appropriate as the amplification factor due to Japanese MBM.



4.2.5 Method of converting MBM and animal fat to cattle

When calculating contamination risk, an infection unit is needed in order to uniformly express the infectivity of different media (live cattle, MBM, animal fat). Infected cattle are in units of single animals, which are easy to handle as integers, and provide the starting point for all contaminant substances. Therefore, we decided to make our basic risk unit 1 infected animal, and converted MBM and animal fat back into an infected animal equivalent for our calculation. According to the EU's geographical BSE risk assessment (GBR), the average weight of MBM taken from one animal is estimated at 65kg. Meanwhile, the volume of animal fat produced from a single animal is the sum of fancy tallow produced from organ fats of slaughtered cattle, and fats (mainly for feed, yellow grease) produced when manufacturing MBM. These are multiplied by their respective rendering yield ratios to produce the total animal fat volume per animal of (65kg x 0.7) + (135 kg x 0.1) = 59kg.

MBM and Animal Fat Conversion Equation

1) In the EU's GBR, the average MBM volume per animal is estimated at 65kg, and this is thought to be an appropriate figure.

2) Volume of fat produced per animal:
   - Fat (fancy tallow) produced from carcass fat65kg x 0.7 (rendering yield ratio) = 45.5kg
   - Fat produced from bone and other inedible parts(mainly for feed, yellow grease)135kg x 0.1 (rendering yield ratio) = 13.5kg

3) Total fat volume: 45.5kg + 13.5kg = 59kg

4.2.6 Rationale on scale of occurrence, probability, and reliability

4.2.6.1 Rationale on scale and probability of exposure to BSE-infected cattle

The rationale on infectivity used in the calculations above can be summarized as follows.

(1) The basic risk unit is 1 contaminated animal. Live cattle, MBM, and animal fat are all converted back into a contaminated animal equivalent. The infectivity per animal is 8,000 infectious units (ID_{50}).

(2) The amplification factor through MBM from 1 contaminated animal is a multiple of 3-6 times, the same as in France and Ireland, with a maximum probability of 4 times used in calculations. The detailed rationale for this is as follows. The infectivity decreases to 1/2000, based on decreases due to the MBM manufacturing process and the susceptibility of calves. For example, about half of BSE-contaminated cattle are sent to slaughterhouses to be processed for meat between the ages of about 24 and 30 months, when the BSE agent is not detected. Therefore, the augmentation factor here is 1/2. The infection value reduces by 1/10 due to boiling and other processes, 1/10 due to drying and agglutination, 1/10 due to the susceptibility of calves, and so on. In this way, the amplification factor through MBM is usually 4 times (3-6 times) for 1 contaminated animal (8,000 ID_{50}).

(3) The rate of decrease in infectivity due to the animal fat manufacturing process is 10^{-6}. This is calculated as the product of cattle sent to slaughterhouses to be processed for meat (1/2, as above), the risk of cross-contamination arising when recovering fat in slaughterhouses (2/100), and prion inactivation, i.e. alkali treatment (1/1000) and filtration (1/10) in the manufacturing process. Also, the susceptibility of newborn cattle is thought to be 10 times that of calves. Therefore, the amplification factor for infection through animal fat is 8/100 times (6/100-12/100 times) for 1 infected animal (8,000 ID_{50}).

Rationale on Infectivity for BSE Cattle

1) Live cattle, MBM, and animal fat are all converted back into an animal equivalent (infectivity per animal: 8,000 units of ID50).

2) Amplification factor through MBM from 1 infected animal: 3-6 times (average 4 times used in calculations; infectivity decreases to 1/2000).

3) Decrease in infectivity due to the animal fat manufacturing process: 10-6. Correction for susceptibility of newborns 10 times that of calves, amplification factor 8/100 times.

Infectivity per animal: 8,000 ID50 → **Decrease in infectivity due to MBM manufacturing process x calf susceptibility: 1/2000** → **MBM amplification= 4**

Decrease in infectivity due to the animal fat manufacturing process: 10-6. Susceptibility of newborns: x 10 → **Animal fat amplification= 8/100**

4) Scale of occurrence and probability of occurrence are treated separately.

MBM and Animal Fat Conversion Equation

4.2.6.2 Rationale on scale of occurrence, probability of occurrence, and reliability

In this analysis, the scale of occurrence is shown as the product of the amplification factors at each stage. Probability is shown as the product of the susceptibility of phenomena at each stage. While the amplification factor and probability are products of the various phenomena, reliability is taken as the average susceptibility of each phenomenon (in other words, a guide to the average reliability of a scenario calculated by dividing the sum of probabilities by the number of phenomena). In this analysis, moreover, scenarios with an occurrence of 0.05 cattle or less, a probability of 0.1 or less, or a reliability of 30% or less are discarded as unrealistic, with the exception of introduction risk scenarios.

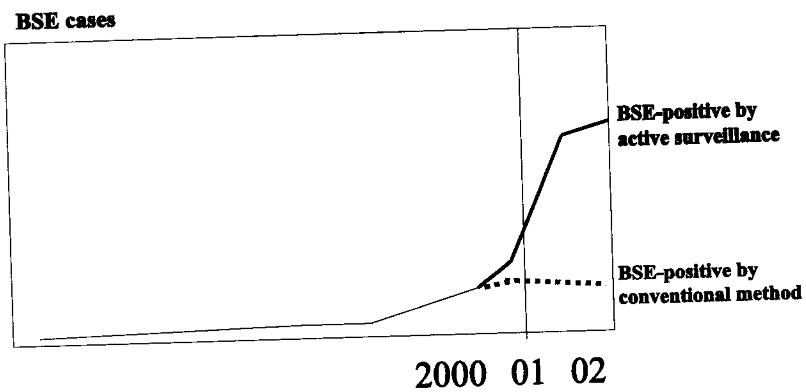
The estimated number of infected cattle in a herd in which one confirmed BSE case exists is based on the ratio between the EU's published numbers of cases identified through active surveillance from 2000 onwards, and trends in the numbers estimated to have been identified by conventional passive surveillance. The ratio between the estimated AUC (area under the curve) of passive surveillance in 2001 and 2002 and the AUC of active surveillance in the same year is around 4 times. Therefore, we may predict a distribution in which the estimated number of infected cattle in a herd with 1 confirmed case peaks at 4. In terms of probability, 3 cattle would be weighted at 0.1, 4 at 0.8, and 5 at 0.1.

If a single infected animal enters rendering and the infection is amplified, the amplification factor would be 3-6 times, as in the EU, with a predicted median of 4. In terms of probability, 3 times would be weighted at 0.1, 4 times at 0.7, 5 times at 0.15, and 6 times at 0.05.

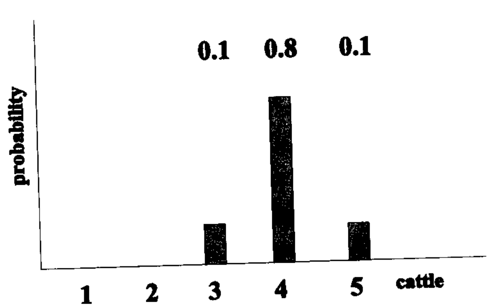
The spread of infection due to animal fat is treated similarly. The infection amplification of 1 infected animal due to animal fat is 6/100, 8/100, and 12/100. In terms of probability, 6/100 times is weighted at 0.1, 8/100 times at 0.8, and 12/100 times at 0.1.

Therefore, the scale, probability and reliability if a herd including 1 confirmed case enters rendering and the infection is amplified would be 3-5 infected animals and an infection amplification of 3-6 times by rendering. In other words, the minimum scale would be 9 cattle (probability 0.01), the median value would be 16 cattle (probability 0.56), and the maximum scale would be 30 cattle (probability 0.005). The reliability is shown as an average percentage of the probability of each phenomenon. The reliability of 9 cattle (minimum) would be 10%, that of 16 cattle (median) 75%, and that of 30 cattle (maximum) 7.5%.

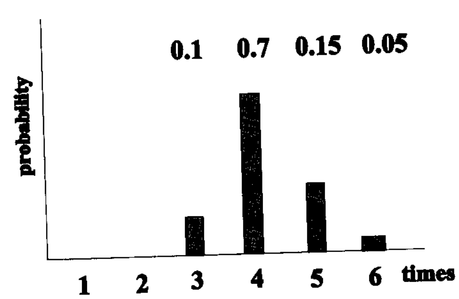
Comparison between BSE-positive cattle found in active surveillance in the EU and the detection frequency of BSE cattle using the conventional method



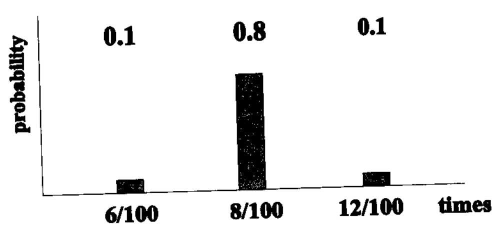
Probability of infected cattle in a herd including 1 confirmed case Probability



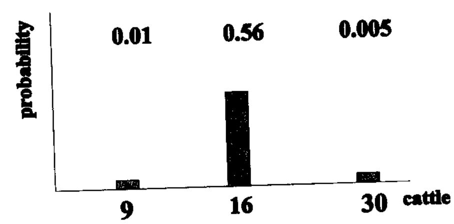
Amplification through MBM from 1 infected animal



Amplification through animal fat from 1 infected animal



Scale and probability if a herd including 1 confirmed case enters rendering and infection is amplified



4.2.6.3 Scenarios of Japanese cattle exposed to contaminated imported MBM (Mø)

While the exposure of domestic cattle due to the import of infected live cattle is relatively easy to calculate, the case of imported MBM or animal fat is more complicated. Below, we take up the example of MBM to explain the rationale.

(1) Assuming the import of A tons of MBM to Japan in the target period (e.g. 5 years), the animal equivalent will be A x 1000 ÷ 65 (kg) = B animals

(2) Theoretical number of cases among cattle in the exporting country

- Assuming the UK exports C live animals to the exporting country in the target period, and the incidence risk in the year of birth is d%, the number of cases will be C x 0.0d = E animals.
- Assuming the UK exports F tons of MBM to the exporting country in the target period, the animal equivalent is F x 1000 ÷ 65 (kg) = G animals, and the incidence risk among UK cattle in the target period is f%, the number of cases will be G x 0.0f = H animals.
- The number of cases among domestic cattle in the exporting country during the target period is taken as J animals.

(3) The total incidence risk number of cattle in the exporting country during the target period will be E + H + J = K. Since the number of infected cattle is thought to be 3-5 times the number of confirmed cases, S will be (Kx3, Kx4, Kx5) animals and P will be (0.1, 0.8, 0.1).
Note: S indicates the size of the infection risk (number of animals).
P indicates the respective probability of each size.

(4) Assuming 1 million cattle per year are processed in slaughterhouses in the exporting country, the total in 5 years will be 5 million. Therefore, the risk level of MBM imported into Japan will be as follows, in animal equivalent conversion:
S = (Kx3, Kx4, Kx5) animals ÷ 5 million x B, and P = (0.1, 0.8, 0.1).
In fact, if S is (C x 0.0d + F x 1000 ÷ 65 x 0.0f + J) x 3 ÷ 5 million x A x 1000 ÷ 65 animals, P is 0.1.
If S is (C x 0.0d + F x 1000 ÷ 65 x 0.0f + J) x 4 ÷ 5 million x A x 1000 ÷ 65 animals, P is 0.8.
If S is (C x 0.0d + F x 1000 ÷ 65 x 0.0f + J) x 5 ÷ 5 million x A x 1000 ÷ 65 animals, P is 0.1.

(5) The imported MBM (Mø) is consumed as feed by domestic cattle within 1 year. It is estimated that, 5 years after this, domestically infected cattle (MøB) will manifest the disease due to Mø. Since the amplification due to MBM is thought to be 5-6 times, S at that time will be (Kx12, Kx16, Kx20) and P will be (0.15, 0.56, 0.17).

(6) If the domestically infected cattle (MøB) are recalled, they are expected to become new sources of infection in the form of MøBM (MBM) and MøBF (animal fat), thereby amplifying the infection.

4.3 Factual background

4.3.1 BSE incidences in the UK

The diagram below shows the BSE incidence rate per year (1986-2001) based on numbers of BSE-infected cattle in the UK, according to the BSE Progress Report published by the UK's Department for Environment, Food, and Rural Affairs (DEFRA) in December 2001. BSE first broke out in the Cornwall area in 1986, before spreading to the whole of England and Wales in the following year. In 1988, it reached Scotland. The incidence rate started to rise in areas around

Canterbury, Brighton and Reading, and reached 0.5-1% in nearly all parts of England in 1990. The outbreak peaked in 1992, when the incidence rate was 0.2% in the Scottish Highlands and 0.3% or less in the rest of Scotland, 1% in Wales and Central England, 1.5% in Yorkshire and Lancashire, and 1.5% or more in Norfolk, Sussex, Cornwall and other parts of southern England.
After peaking, BSE incidences gradually declined from the north downwards. By 1995, the incidence rate had reached 0.2% throughout Scotland and Wales, and in the whole of England except Norfolk by 1998. The average incubation period of BSE until clinical manifestation is thought to be 5 years (ranging from 2 to 8 years). Therefore, we determined the year in which the confirmed cases were born, by subtracting 5 years from the years in this diagram.

BSE Incidences in the UK

BSE incidence rate based on numbers of BSE-infected cattle in the UK (DEFRA, BSE Progress Report, December 2001)

4.3.2 BSE incidences in other countries

The following table shows BSE incidences in various other countries between 1989 and 2001. Unlike the UK, there was no explosive outbreak in these. They divide into countries such as France, Ireland, Portugal and Switzerland, where there were significant incidences of BSE from quite an early stage, and others in which incidences started in around 2000. Japan is thought to be closer to the latter group. As will be discussed later, the numbers and years of BSE incidences in Italy, the Netherlands, Denmark, Switzerland and Germany will also be involved as data for the introduction risk, besides those of the UK.

	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	Total
Austria	0	0	0	0	0	0	0	0	0	0	0	0	1	0	...	1
Belgium	0	0	0	0	0	0	0	0	1	6	3	9	46	38	11	114
Canada	0	0	0	0	1(b)	0	0	0	0	0	0	0	0	0	1	2
Czech Republic	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	5
Denmark	0	0	0	1(b)	0	0	0	0	0	0	0	1	6	3	2	13
Finland	0	0	0	0	0	0	0	0	0	0	0	0	1	0	...	1
France	0	0	5	0	1	4	3	12	6	18	31(a)	161	274	239	93	847
Germany	0	0	0	1(b)	0	3(b)	0	0	2(b)	0	0	7	125	106	9	253
Greece	0	0	0	0	0	0	0	0	0	0	0	0	1	0	...	1
Ireland	15(a)	14(a)	17(a)	18(a)	16	19(a)	16(a)	73	80	83	91	149	246	333	104	1274
Israel	0	0	0	0	0	0	0	0	0	0	0	0	0	1	...	1
Italy	0	0	0	0	0	2(b)	0	0	0	0	0	0	48	38(a)	...	88
Japan	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	7
Liechtenstein	0	0	0	0	0	0	0	0	0	2	...	...	...	...	...	2
Luxembourg	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2
Netherlands	0	0	0	0	0	0	0	0	2	2	2	2	20	24	8	60
Poland	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	6
Portugal	0	1(b)	1(b)	1(b)	3(b)	12	15	31	30	127	159	149	110	86	62	787
Slovakia	0	0	0	0	0	0	0	0	0	0	0	0	5	6	1	12
Slovenia	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	3
Spain	0	0	0	0	0	0	0	0	0	0	0	2	82	127	99	310
Switzerland	0	2	8	15	29	64	68	45	38	14	50	33	42	24	14	446
UK	7228	14407	25359	37280	35090	24438	14562	8149	4393	3235	2301	1443	1202	1,144		183191*

* : includes some in or before 1988

(a) ***France:*** includes 1 incidence among imported cattle (decided on 13/08/1999)
Ireland: includes incidences among imported cattle (5 in 1989, 1 in 1990, 2 in 1991, 1 in 1994, 1 in 1995)
Italy: includes 2 incidences among imported cattle
Portugal: includes 1 incidence among imported cattle (decided on 22/11/2000)

(b) Incidences among imported cattle

BSE incidences in various countries (OIE report, August 2003)

4.3.3 Live cattle imported from the UK

The following table shows imports of live cattle from the UK by various countries. In EU countries, we may postulate 3 patterns for the introduction of the BSE agent. The first postulate is that Ireland and Portugal directly imported large numbers of infected cattle, and the BSE agent derived from these cattle was amplified through rendering in their own countries. Next, it is thought that the import of contaminated MBM, rather than infected cattle, caused BSE contamination in France and Belgium. On the other hand, even Switzerland has been affected by BSE, even though, statistically, it has imported no infected cattle or contaminated MBM from the UK at all. This is thought to be due to the introduction of BSE agent through EU countries other than the UK. In this period, Italy and Denmark (from which Japan imported large volumes of MBM) and the Netherlands (likewise, animal fat) imported live cattle from the UK. Finally, Canada imported 160 live cattle from the UK during this period.

Numbers of Live Cattle Imported from the UK								
	1985	1986	1987	1988	1989	1990	1991	1992
France	243	124	132	158	271	178	1,155	2,623
Denmark	112	222	212	213	130	18	0	0
Italy	115	357	305	244	400	179	71	0
Ireland	10,917	4,742	4,365	6,459	4,666	1,293	261	820
Netherlands	110	86	955	117	166	1,906	0	5
Germany	498	789	1,403	2,265	1,388	715	3	11
Portugal	3,840	2,253	2,831	1,108	656	0	0	0
Spain	1,092	795	252	277	353	0	0	63
Belgium	110	189	80	95	98	41	0	99
Greece	0	7	0	0	0	0	0	0
Czech Republic				0	0	0	0	0
Switzerland								
USA	Average 50 per year							
Canada	Imported about 160 beef cattle from the UK between 1982 and 1990.							
Japan			9	19				

4.3.4 Incidences of BSE in Japan and the investigation into the source of infection

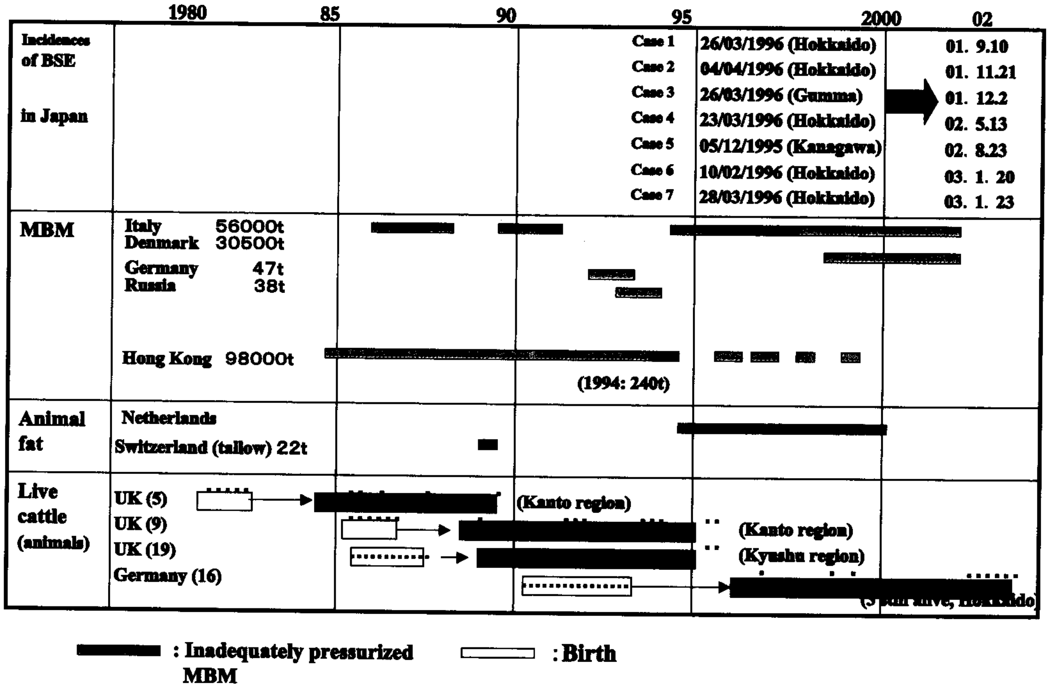
So far, 7 cases of BSE infection have been confirmed in Japan. All of these animals were born between December 1995 and April 1996, and manifested the disease between 2001 and 2003. Here, we will consider what kinds of infection source could have introduced BSE into the country, and by what route.

Japan imports relatively large quantities of MBM from Italy. Up to 1988, this MBM was subjected to inadequate pressure and heating in the manufacturing process. After 1998, however, measures were taken to reduce infectivity by changing the MBM manufacturing method (133°C, 3 bar, 20 minutes). MBM is also imported from Denmark, Germany and Russia, while the possibility cannot be ruled out that UK-produced MBM was also imported via Hong Kong. Besides these, bone meal has been imported from the UK as well as from Ireland, where BSE contamination was high from the early stages. However, we have shown that this was edible bone meal used mainly as a source of calcium, and have therefore created no risk scenario for it.

Next, animal fat has been imported from the Netherlands (powdered fats) and Switzerland. It is possible that these were fed to Japanese cattle as an ingredient in milk replacer.

Finally, 49 live cattle were imported from Europe – 5 from the UK in 1982 (destination: Kanto region), 9 from the UK in 1987 (Kanto), 19 from the UK in 1988 (Kyushu), and 16 from Germany in 1993 (Hokkaido) – in addition to 5,210 from Canada. Owing to a paucity of data concerning the risk from Canadian live cattle or MBM, these are not handled in this scenario. They will need to be analyzed on the basis of future investigation data. The following chart summarizes the results of the infection source investigation to date.

Incidences of BSE in Japan and Investigation into the Source of Infection



4.3.5 Imported live cattle and the risk derived from them

The risk of the BSE agent being introduced to Japan through imports of live cattle (all dairy cattle) can be divided into four scenario groups. These are the 5 cattle born in southern England and imported into the Kanto region in 1982, the 9 cattle born in southern England and imported into the Kanto region in 1987, the 19 cattle born in southern England and imported into Kyushu in 1988, and the 16 cattle born in Germany and imported into Hokkaido in 1993. The risk from imported German cattle exists in 5 animals, excluding 8 that proved negative in BSE tests and 3 that are still alive.

Live cattle are easier to investigate than MBM or animal fat, since the history of each animal is known. On studying the respective import lots, we find that, over time, the origin of live cattle imports from the UK (all dairy cattle) gradually shifted from central to southern England, where BSE contamination was more intense. This study also clarifies issues such as where the cattle were reared in Japan after import, slaughterhouse processing after exhaustion of the reproductive cycle, MBM manufacturing processes, and where the cattle were re-used as MBM. We conducted risk analysis by adding such data to the testing system in use at the time, the state of use of MBM, the level of BSE incidence in the UK at the time of import, and other factors. We then made risk predictions, and investigated the relative consistency of these models with actual facts. We conducted similar risk analysis for imported MBM and animal fat as for imported live cattle.

Risk from imported live cattle

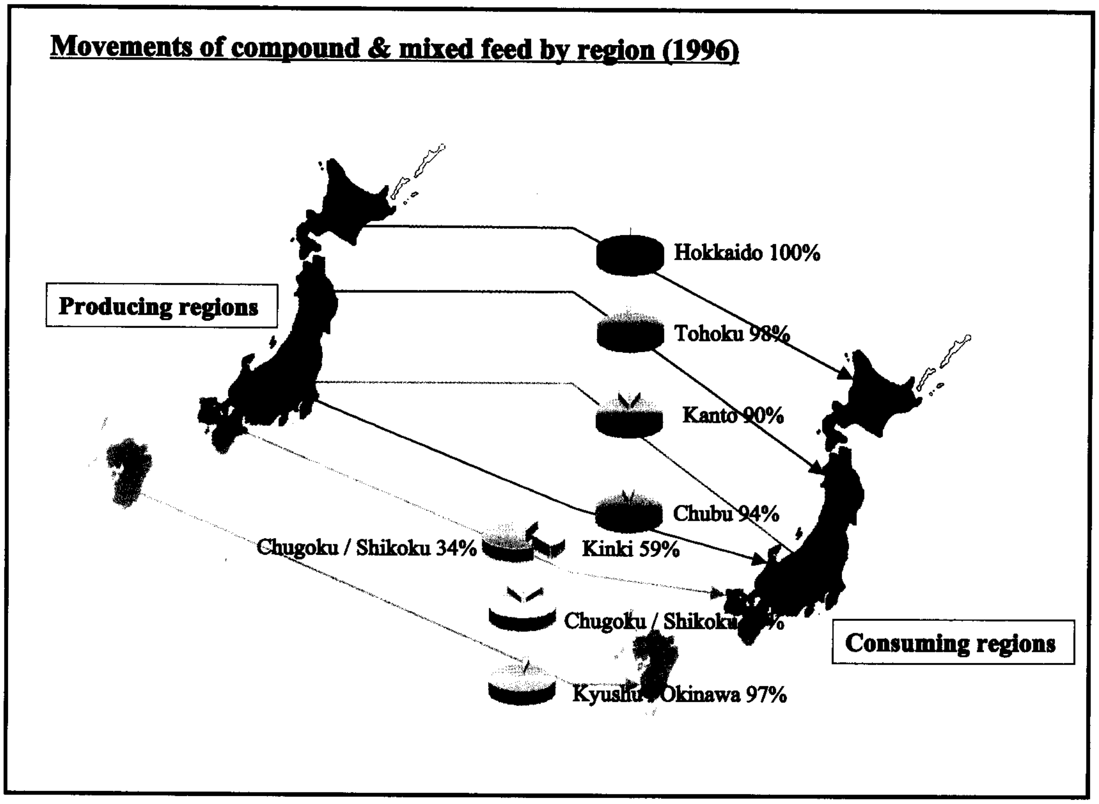
	UK 5 cattle, 1982	UK 9 cattle, 1987	UK 19 cattle, 1988	Germany 16 cattle, 1993
Place of birth	Central England	Central-southern England	Southern England	?
Date of birth	1979-1980	Oct. 1985 – Mar. 1986	Sept. 1985 – Sept. 1986	Apr. 1991 – Sept. 1993
Termination Recalled Culled Died Still alive	3-9 years old(1984-89) 5 cattle	4-10 years old (1989-93) 8 cattle 1 cattle	3-10 years old (1989-95) 17 cattle 2 cattle	5-11 years old 3 cattle (8 negative in BSE tests) 3 cattle 3 cattle
Import destination	Kanto	Kanto	Kyushu	Hokkaido
Still alive / slaughter / rendering	Kanto (1984-85)	Kanto (1992-93) 3 slaughterhouses, 2 rendering plants	Kyushu (1989, 1992, 1993) 1 slaughterhouse, 2 rendering plants	Hokkaido(2002) 1 slaughterhouse, 2 rendering plants
State of recalled cattle	Reproductive disorders Postnatal astasia Decreased milk yield Displacement of abomasum External injury	Recalled due to decreased milk yield. All culled cattle were incinerated after BSE testing (negative). No cattle displayed nervous signs.	Reproductive disorders, mastitis, others.2 cattle died from acute pneumonia and ketosis.	Old age (10-11 years) 3 cattle died from fatal fall, mastitis, and piroplasmosis.
MBM Yellow grease	Kanto	Kanto	Kyushu, Shikoku	Hokkaido, Tohoku
Animal fat	East Japan	East Japan	West Japan	East Japan

4.3.6 Movements of compound & mixed feed by region (1996)

The following diagram shows relative movements of compound and mixed feed within Japan, by region. It reveals that compound and mixed feed for cattle is basically produced and consumed within the same region.

Feed produced in Hokkaido is consumed almost 100% within Hokkaido. If we postulate that MBM produced in Hokkaido was contaminated, the contamination would have stayed within Hokkaido and the possibility of it spreading to other regions is virtually nil.

Feed produced in other regions is consumed as follows. Of feed produced in Tohoku, 98% is consumed within Tohoku. Of feed produced in Kanto, 90% is consumed in Kanto, and 5% each in Tohoku and Chubu. Of feed produced in Chubu, 94% is consumed in Chubu, 3% in Kinki and 2% in Kanto. Of feed produced in Kinki, 59% is consumed in Kinki, 34% in Chugoku and Shikoku, and 6% in Chubu. Of feed produced in Chugoku and Shikoku, 84% is consumed in Chugoku and Shikoku, 9% in Kinki and 7% in Kyushu and Okinawa. Of feed produced in Kyushu and Okinawa, 97% is consumed within those regions. In other words, production and consumption are almost invariably within the same region. The only notable exception is feed produced in the Kinki region, which is shipped to Chugoku and Shikoku.



Movements of compound & mixed feed by region

4.3.7 Sales destinations of MBM derived from imported live cattle

Most imported live cattle are thought to be processed as ingredients for MBM or animal fat at the age of 3 or older. MBM and animal fat derived from the 5 live cattle imported from the UK to the Kanto region in 1982 (C1) are thought to have been sold in the Kanto region. It is conceivable that MBM and animal fat derived from the 9 live cattle imported from the UK to the Kanto region in 1987 (C2) were sold in Kanto, and that MBM and animal fat derived from the 19 live cattle imported from the UK to Kyushu in 1988 (C3) were sold in the Kyushu and Chugoku regions. Of the 16 live cattle imported from Germany to Hokkaido in 1993 (C4), MBM and animal fat derived from 5 cattle (excluding 8 that proved negative in BSE tests and 3 that are still alive, as of August 2003) are thought to have been sold in Hokkaido and the Tohoku region.

In the Japanese livestock industry, Hokkaido produces mainly dairy cattle and Kyushu mainly beef cattle. As such, the exposure risk for domestic cattle is not necessarily uniform. It is highly likely that MBM produced in Kyushu was mainly used as compound feed for swine and poultry farming. When MBM from rendering is only used at low frequency for feeding cattle, it is highly likely that the subsequent exposure risk and amplification factor due to MBM will be lower than the theoretical value. However, since there is a lack of accurate data, we took no account of these points in this scenario.